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Research Article

Dynamics of Malondialdehyde Depending on Cadmium Concentration and their Relation with the Daily Weight Gain on the Chicken Hybrid

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Abstract

Objective: The aim of this study was to determine the effect of cadmium (Cd) on the level of phospho-lipids peroxidation, by measuring the level of malondialdehyde (MDA) and study the changes on body weight of egg-laying breed chickens. **Methodology:** Total four groups of chickens have been included in the experiment. Three groups treated with different concentrations of cadmium (2, 4 and 6 mg kg⁻¹ of live weight added in drinking water) and one group as a control without treatment with Cd. The data were analyzed statistically by using SPSS software package. ANOVA test and the medium and standard error of the mean were presented. **Results:** The result shows that, level of malondialdehyde in blood is increased by increasing the cadmium on daily diet of chicken. On the control group, the MDA level was 17 µmol L⁻¹ when we have treat with cadmium 2 mg kg⁻¹ the level increase amounts twice (30 µmol L⁻¹), on the 3rd group 44 µmol L⁻¹ when was supplemented with 4 mg kg⁻¹, while on the 4th group (supplemented with 6 mg kg⁻¹ Cd) it reach up to 64 µmol L⁻¹. In another hand, body weight of egg-layer chicken in three different times point, supplemented with different amount of Cd shows to be different. When the chickens was not treated with Cd body weight on last days reach up to 1114 g while by adding the Cd on feed the body weight has decrease to 734 g. **Conclusion:** It can be concluded that, MDA is increase by increasing the cadmium concentration on daily diet. This is due to makes difficulties on peroxidation of the lipids or proteins on body chickens and also plays negative effects on increasing the body weights. Also the body weight of the egg-laying breed chickens was decreased by feeding the chicken with Cd indicating that Cd plays a negative effect on feeds and most probably on the quality of the meat.

Key words: Cadmium, effect of toxic element, egg-laying breed chickens, oxidative stress, malondialdehyde, body weights gain, peroxidation of phospholipids

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cadmium (Cd) is known to be a toxic heavy metal that poses a hazard to animal health due to its toxicological effects^{1,2}. Based on the effect of the toxicity, the cadmium can be found in different types and can be accumulate in human and animal body. The most toxic type of cadmium salt is the cadmium oxide, less toxic is cadmium sulfate whereas the cadmium chloride occupies an intermediary position^{3,4}. The permissible dosage of cadmium is varied differently in human and animal body. Even, these differences varied differently on diet and concentrated in different organs and amounts⁵. In the egg-laying breed chickens the likelihood of cadmium toxicity can be assessed (as normal, high and toxic range) from cadmium concentration in the diet, kidney and liver and the level of cadmium as a toxic is $>40 \text{ mg kg}^{-1} \text{ DM}^6$. The influence of cadmium in the metabolism of calcium in the osseous tissue accompanied with the emergence of osteomalacia and remodeling of osseous tissue⁷. This reaction is more abundant when the cadmium applied for at least 6 months in dosage of 25 mg L^{-1} of drinkable water^{8,9}.

A study carried out by Watjen and Beyersmann¹⁰ determined that cadmium ions have direct impact on the metabolism of calcium and structure of osseous tissue. Another mechanism of reaction of cadmium ions is supposed to be the decrease of calcium absorption from the digestion apparatus because of impediment of activation of D_3 vitamin in the renal cortex¹¹. Cadmium ions have an impact in the growth of peroxidation processes that leads to impediment of growth rhythm) points out that the effect of cadmium ions in impediment of the growth is proportional to the cadmium intake amount on daily basis¹². Previously was points out that the cadmium disposal at the amount of 100 ppm causes slight impediment in increase of live weight, biochemical and hematological changes, kidney damage, bursa of fabricius, liver and osseous tissue. Growth of free radicals in proportion to the cadmium intake dosage causes not only lipid peroxidation which contain insatiable fatty acids and highly insatiable¹³. In this way the growth of concentration of malondialdehyde (MDA), this also causes peroxidation of deoxiribonucleic which in turn cause damage of DNA structure¹⁴⁻¹⁶.

The aim of this study was to determine the level of peroxidation of phospho-lipids, by measuring the level of malondialdehyde and its impact on body weight of egg-laying breed chickens using cadmium.

MATERIALS AND METHODS

Four groups of chickens ("Isa Brown" hybrid) have been included on experiment. Each group contains six egg-laying breed chickens. All chickens were placed in cages two by two per one cage. Egg-laying breed chickens were treated for a month in the preparatory period with the same food portion and were weighed at the end of this period. Following completion of this procedure, the experimental period commenced. The first group was selected as a control group, the 2nd group was treated with cadmium chloride supplement of 2 mg kg^{-1} cadmium of live weight, the 3rd group was treated with 4 mg kg^{-1} cadmium and the 4th group was treated with 6 mg kg^{-1} cadmium of live weight.

This treatment continued for a month when the second weighing took place. After the period of another 25 days, the third weighing took place. The whole experimental period continued for 55 days. Food treatment was the same for four groups during the experimental period. Based on the weighing data, the difference between the first weighing and the second weighing was calculated, as well as the difference between the second weighing and the third weighing. On the 55 days of experimental period, we have analyzed the level of MDA in the blood of the "Isa Brown" hybrid by using spectrophotometer at the Faculty of Agriculture and Veterinary at the University of Pristina. Working procedure is performed based on kit guidance (BIOXYTECH[®] MDA-586[™]). In brief for each sample is taken a tube, where in each tube is placed $10 \mu\text{L}$ of probucol, $100 \mu\text{L}$ of blood plasma (sample) and $640 \mu\text{L}$ of reagent R1. Then, after we mixed the samples and we added $150 \mu\text{L}$ of second reagent R2 and incubated at 45°C for 60 min followed by centrifugation for 10 min at $10000 \times g$ until the supernatant obtained clean. In the end, the supernatant is transferred via automatic pipets to the spectrophotometric UV-Vis (SECOMAM, Anthelie advanced 5) kuvets and the measurements were done on 586 nm wavelength.

The obtained data were analyzed statistically by using ANOVA test processed by determining for each group the medium and standard error of the mean (SEM), the validation of the MDA level change among groups, including the coefficients of correlation between the MDA level and daily live weight gain, including the data of difference of weight gain between the first weighing and second weighing, as well as between the second weighing and third weighing.

RESULTS

Previously is known that, reactive oxygen species deplete polyunsaturated lipids, forming malondialdehyde which is a reactive aldehyde and it can cause toxic stress in cells and form covalent proteins adducts referred to as advanced lipoxidation end-products⁵. The MDA level represents a level increase in addition to the increase of supplemented cadmium chloride on egg-laying breed chicken under growth. On the control group, the MDA level was 17 $\mu\text{mol L}^{-1}$, while in the 2nd group the level increase amounts twice (30 $\mu\text{mol L}^{-1}$), on the 3rd group 44 $\mu\text{mol L}^{-1}$, while on the 4th group it reach up to 64 $\mu\text{mol L}^{-1}$. These results are presented in Table 1.

Regarding to coefficient of variation it is observed that the diversity of individuals is low in the first group (CV = 1.1), whereas in the experimental groups the diversity of each individual group is higher (variation from 6.5-9.5%). These data prove that the reaction among chickens is not the same against the level of cadmium ions taken through gastrointestinal apparatus.

The values from Table 2 represent the data when we have measured the body weight of the egg-layer chickens in three different times point and different cadmium concentration. The results show that, body weight of chickens goes up from 300 g to approximately 1200 g without supplemented with

cadmium while on the samples treated with cadmium is lower. The result shows that, as higher the cadmium concentration is added on the chickens the body weight is lower.

The differences between the first groups and other groups, including the validation of difference between them indicates that in the beginning of the experiment the chicken's body weight was statistically the same while the difference of the live weight in the first measurement among these groups represented differences with high statistical validation. These data support the dynamic that was determined in the experimental study. These results prove that the dynamic of live weight gain at the end of preparatory period has no statistically validated changes between the first group and three other experimental groups. However, as far as the changes at the third stage of the experiment are concerned, it can be observed that changes were statistically validated for a high level ($p > 0.001$). Previously it was reported that, correlation between the weight gains on chickens does not represent any significant correlation with the MDA level on blood¹⁷. This is in agreement with our finding.

In Table 3 are shown the body weight differences between different treatments presented on percentage. The result shows that, the body weight was decreased from first measurement to the end (approximately 83% first measurement while 64% in the third measurement).

Table 1: Dynamics of MDA concentration in blood depending on the cadmium chloride dosage expressed in $M \pm SEM$ (n = 6)

Measurement	Egg-layer chickens groups			
	I	II	III	IV
MDA ($\mu\text{mol L}^{-1}$)	17.62 \pm 0.19 ^a	30.26 \pm 2.78 ^b	44.32 \pm 4.2 ^c	64.01 \pm 6.5 ^d
CV	1.1	9.2	9.5	6.5

Table 2: Chickens body weight gain depending on the supplementation with cadmium expressed in gram

Replicate	Groups (g)			Differences		Daily gain	
	I	II	III	I with II	II with III	1:2	2:3
I	300 \pm 29.57	861 \pm 44.7	1114 \pm 44.7	561 \pm 17.6	292.1 \pm 17.6	18.68 \pm 0.6	11.76 \pm 1.35
CV	9.8	5.2	11.7	3.1	3.1	3.1	11.6
II	250.6 \pm 19.4	742 \pm 78.5	968 \pm 35.5	490.8 \pm 19.3	226 \pm 15.3	16.4 \pm 0.65	9 \pm 0.61
CV	7.7	4.7	3.7	3.9	1.8	4	6.8
III	248.2 \pm 15.4	727.8 \pm 37.9	838 \pm 37.1	479 \pm 32.7	110 \pm 31.1	16 \pm 1.1	2.91 \pm 2.8
CV	6.2	5.2	4.4	6.8	2.8	6.8	9.8
IV	226.5 \pm 12.9	663.8 \pm 49.7	734 \pm 72.6	436 \pm 49.5	72.3 \pm 19.9	14.5 \pm 1.65	2.9 \pm 0.788
CV	5.7	8.5	9.9	11	2.7	11.4	27.2

M \pm SEM and CV: Coefficients of variation

Table 3: Live weight of chickens during three measuring occasions according to the groups expressed in percentage (first group is considering 100%)

Groups (%)	II with I	III with I	IV with I
I	83.5	82.7	75.0
II	86.1	84.5	77.1
III	84.8	73.4	64.3

DISCUSSION

In the present study level of MDA was measured on egg-laying breed chicken when different concentration of cadmium (2, 4 and 6 mg kg⁻¹) was supplemented on feed. The final product of polyunsaturated fatty acids peroxidation in the cells is known to be MDA^{18,19}. Overproduction of MDA will come by increasing the free radicals and frequently is used as a marker of oxidative stress and the antioxidant in cancerous patients²⁰. In another hand, the toxic effect of heavy metals are reported especially cadmium which has been shown to be one of the most toxic metals in environment^{21,22}. From gastrointestinal tract Cd was absorbed on the kidney and liver where this element binds to metallothionein (MT) and by blood can cause toxicity in various tissues and organs²³. In this experiment we treated the egg-layer chicken with different Cd concentration supplemented on feed and level of MDA was measure in the end of the experiment. The result shows that, that by increasing the feed with Cd, level of MDA significantly increase compare with control at the end of the study period (Table 1). In previous study by Liu *et al.*²⁴ shows increase the level of cadmium cause high toxicity and most of the accumulation of this element was on kidney causing apoptosis.

More study showing the effect of cadmium in MDA level has been shown also in other animals such as in rat²⁵ showing that Cd accumulation is different in various organs with highly accumulated on kidney and manifesting different disease²⁴. By increasing the body weight the oxidative stress is caused. In line with that are several studies which has promoted also previously showing that Cd promote oxidative stress by causing mitochondrial dysfunction on kidney cells^{25,26}. While, Hagar and Al Malki²⁷ reported that cadmium treatment reduced antioxidative activity in rats. In another studies shows oxidative damage is cause when the Cd is high in body of mice and induced renal apoptosis^{28,29}. This indicating that cadmium led to renal oxidative damage and induced renal apoptosis in mice and also decrease the MDA level. In contrast from our result, other studies show that level of lipid peroxidation was decreased by adding Cd^{30,31}. This probably due to the different organs accumulates cadmium differently (liver, kidney and testis) after chronic exposure^{32,33}. When the levels of MDA from different organs are differently is necessary to measure MDA level on blood is more adequate than on other specific organs. In the current study, we also found that Cd significantly decreased the body weight of the egg laid chicken significantly by adding high amount of Cd on feed. Interestingly some of the previous data are in disagreement compare to our result. Erdogan *et al.*³⁴ has reported that, when animals was offered limited feed or low

quality of feed or when the quality of feed was low and degradation of lipids and proteins are increased as a result of cadmium toxicity and the level of cadmium accumulation was higher for long time in body weight the animals become chronic toxicity manifested by different disease in human and animal body³⁵. Our result presented on Table 2 shows significant differences by increasing the level of supplemented Cd incorporated with water in higher amounts and analyzed between contaminated and controls egg-laying breed chickens.

Interestingly, in rates when the Cd was supplemented on feed, the feed wasn't consumed from rates and in same time the body weight of them was decreased which is in contrast compared from our result when the water supplemented with Cd was consumed fine. This is probably due to the importance of water in human and animal body. When different heavy metals were add in chickens and experiment was performed for 8 weeks it is observed a decrease in the dynamic of weight gain depending on the dosage of supplemented cadmium on diet³⁶. At the end of 8 weeks' period, the researchers determined that the live weight amounts to 2230 g in the control group whereas the live weight amounts to 1070 g in the group with 30 ppm. These data are in agreements with our result and we conclude that heavy metals especially Cd adding on the feed plays a role in decreasing the performance of body weight of the chickens. When we transfer the data in percentage, the results from the horizontal point of view shows that, the group measuring in first time comparing with the control group, the body weight decreases to 83.5% in the 2nd group and up to 75% in the 4th group are presented in Table 3. Same result has been found and during the second and third measuring. While when the data we see on the vertical comparison, we can determine the similar result only the values where low. Also, Bokori *et al.*³⁷ in their studies reported when the cadmium was exposed in chicken the body weight was decreased and every prolongation of dietary cadmium becomes on mortality of the chickens³⁸. Interestingly, our result shows that, no differences on drinking waters between different treatments and different days until the end of experiment was seen. This is probably due to the toxic effects of Cd performance. However, to confirm both hypotheses, explaining how Cd effects on body weight and relation of MDA on different organs and blood further studies on the different concentration of Cd needs to add and MDA to measure on different organs are necessary.

CONCLUSION

The data in this study indicate that level of malondialdehyde in blood increase by increasing

supplemented cadmium chloride in egg-laying breed chickens diet. The MDA is an indication for presence of cadmium. When the level of MDA is higher Cd is present very high in the body weight of the animal and it makes difficulties on peroxidation of the lipids or proteins on body weight of the chickens. By increasing the level of Cd the body weight of egg-laying breed chickens was decrease and the cadmium toxicity is higher even the meat is not allowed to use for consumer.

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REFERENCES

1. Lane, E.A., M.J. Canty and S.J. More, 2015. Cadmium exposure and consequence for the health and productivity of farmed ruminants. *Res. Vet. Sci.*, 101: 132-139.
2. Bosch, A.C., B. O'Neill, G.O. Sigge, S.E. Kerwath and L.C. Hoffman, 2016. Heavy metals in marine fish meat and consumer health: A review. *J. Sci. Food Agric.*, 96: 32-48.
3. Godt, J., F. Scheidig, C. Grosse-Siestrup, V. Esche, P. Brandenburg, A. Reich and D.A. Groneberg, 2006. The toxicity of cadmium and resulting hazards for human health. *J. Occup. Med. Toxicol.*, Vol. 1. 10.1186/1745-6673-1-22.
4. Mah, V. and F. Jalilvand, 2010. Cadmium(II) complex formation with glutathione. *J. Biol. Inorg. Chem.*, 15: 441-458.
5. Chmielowska-Bak, J., J. Gzyl, R. Rucinska-Sobkowiak, M. Arasimowicz-Jelonek and J. Deckert, 2014. The new insights into cadmium sensing. *Front. Plant Sci.*, Vol. 5. 10.3389/fpls.2014.00245.
6. Guzzi, G., P.D. Pigatto and A. Ronchi, 2009. Periodontal disease and environmental cadmium exposure. *Environ. Health Perspect.*, 117: A535-A536.
7. Swandulla, D. and C.M. Armstrong, 1989. Calcium channel block by cadmium in chicken sensory neurons. *Proc. Natl. Acad. Sci. USA.*, 86: 1736-1740.
8. Andersen, O., J.B. Nielsen and P. Svendsen, 1988. Oral cadmium chloride intoxication in mice: Effects of dose on tissue damage, intestinal absorption and relative organ distribution. *Toxicology*, 48: 225-236.
9. Park, S.J., J.R. Lee, M.J. Jo, S.M. Park, S.K. Ku and S.C. Kim, 2013. Protective effects of Korean red ginseng extract on cadmium-induced hepatic toxicity in rats. *J. Ginseng Res.*, 37: 37-44.
10. Watjen, W. and D. Beyersmann, 2004. Cadmium-induced apoptosis in C6 glioma cells: Influence of oxidative stress. *Biometals*, 17: 65-78.
11. Akyolcu, M.C., D. Ozcelik, S. Dursun, S. Toplan and R. Kahraman, 2003. Accumulation of cadmium in tissue and its effect on live performance. *Journal de Physique IV*, 107: 33-36.
12. Shahid, M., B. Pourrut, C. Dumat, M. Nadeem, M. Aslam and E. Pinelli, 2014. Heavy-metal-induced reactive oxygen species: Phytotoxicity and physicochemical changes in plants. *Rev. Environ. Contam. Toxicol.*, 232: 1-44.
13. Uyanik, F., M. Eren, A. Atasever, G. Tuncoku and A.H. Kolsiz, 2001. Changes in some biochemical parameters and organs of broilers exposed to cadmium and effect of zinc on cadmium induced alterations. *Israel J. Vet. Med.*, 56: 128-134.
14. Cinar, M., A.A. Yigit and G. Eraslan, 2010. Effects of vitamin C or vitamin E supplementation on cadmium induced oxidative stress and anaemia in broilers. *Revue Medecine Veterinaire*, 161: 449-454.
15. Cinar, M., E. Yildirim, A.A. Yigit, I. Yalcinkaya, O. Duru, U. Kisa and N. Atmaca, 2014. Effects of dietary supplementation with vitamin C and vitamin E and their combination on growth performance, some biochemical parameters and oxidative stress induced by copper toxicity in broilers. *Biol. Trace Elem. Res.*, 158: 186-196.
16. Kalghatgi, S., C.S. Spina, J.C. Costello, M. Liesa and J.R. Morones-Ramirez *et al.*, 2013. Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in mammalian cells. *Sci. Transl. Med.*, Vol. 5. 10.1126/scitranslmed.3006055.
17. Balogh, K., M. Weber, M. Heincinger, G. Kollar and M. Mezes, 2012. Correlations between daily weight gain, lipid peroxidation and glutathione status of liver and kidney in different pig genotypes. *Archiv Tierzucht*, 55: 263-271.
18. Niki, E., 2015. Lipid oxidation in the skin. *Free Radic. Res.*, 49: 827-834.
19. Grune, T., N. Zarkovic and K. Kostelidou, 2010. Lipid peroxidation research in Europe and the COST B35 action 'lipid peroxidation associated disorders'. *Free Radic. Res.*, 44: 1095-1097.
20. Bayraktar, N., S. Kilic, M.R. Bayraktar and N. Aksoy, 2010. Lipid peroxidation and antioxidant enzyme activities in cancerous bladder tissue and their relation with bacterial infection: A controlled clinical study. *J. Clin. Lab. Anal.*, 24: 25-30.
21. Jarup, L. and A. Akesson, 2009. Current status of cadmium as an environmental health problem. *Toxicol. Applied Pharmacol.*, 238: 201-208.
22. Satarug, S., S.H. Garrett, M.A. Sens and D.A. Sens, 2010. Cadmium, environmental exposure and health outcomes. *Environ. Health Perspect.*, 118: 182-190.
23. Klotz, K., W. Weistenhofer and H. Drexler, 2013. Determination of cadmium in biological samples. *Met. Ions Life Sci.*, 11: 85-98.
24. Liu, L., B. Yang, Y. Cheng and H. Lin, 2015. Ameliorative effects of selenium on cadmium-induced oxidative stress and endoplasmic reticulum stress in the chicken kidney. *Biol. Trace Elem. Res.*, 167: 308-319.

25. Mao, W.P., N.N. Zhang, F.Y. Zhou, W.X. Li and H.Y. Liu *et al.*, 2011. Cadmium directly induced mitochondrial dysfunction of human embryonic kidney cells. *Hum. Exp. Toxicol.*, 30: 920-929.
26. Gamboa, J.L., T. Frederic, I.V. Billings, T.B. Matthew and A.G. Laura *et al.*, 2016. Mitochondrial dysfunction and oxidative stress in patients with chronic kidney disease. *Physiol. Rep.*, Vol. 4. 10.14814/phy2.12780.
27. Hagar, H. and W. Al Malki, 2014. Betaine supplementation protects against renal injury induced by cadmium intoxication in rats: Role of oxidative stress and caspase-3. *Environ. Toxicol. Pharmacol.*, 37: 803-811.
28. Chen, Q., R. Zhang, W.M. Li, Y.J. Niu and H.C. Guo *et al.*, 2013. The protective effect of grape seed procyanidin extract against cadmium-induced renal oxidative damage in mice. *Environ. Toxicol. Pharmacol.*, 36: 759-768.
29. Nair, A.R., O. DeGheselle, K. Smeets, E. van Kerkhove and A. Cuypers, 2013. Cadmium-induced pathologies: Where is the oxidative balance lost (or not)? *Int. J. Mol. Sci.*, 14: 6116-6143.
30. Kawagoe, M., F. Hirasawa, S.G. Wang, Y. Liu, Y. Ueno and T. Sugiyama, 2005. Orally administrated rare earth element cerium induces metallothionein synthesis and increases glutathione in the mouse liver. *Life Sci.*, 77: 922-937.
31. Shibutani, M., K. Mitsumori, S.I. Satoh, H. Hiratsuka and M. Satoh *et al.*, 2001. Relationship between toxicity and cadmium accumulation in rats given low amounts of cadmium chloride or cadmium-polluted rice for 22 months. *J. Toxicol. Sci.*, 26: 337-358.
32. Haouem, S. and A. El Hani, 2013. Effect of cadmium on lipid peroxidation and on some antioxidants in the liver, kidneys and testes of rats given diet containing cadmium-polluted radish bulbs. *J. Toxicol. Pathol.*, 26: 359-364.
33. Haouem, S., M.F. Najjar, A. El Hani and R. Sakly, 2008. Accumulation of cadmium and its effects on testis function in rats given diet containing cadmium-polluted radish bulb. *Exp. Toxicol. Pathol.*, 59: 307-311.
34. Erdogan, Z., S. Erdogan, S. Celik and V. Unlu, 2005. Effects of ascorbic acid on cadmium-induced oxidative stress and performance of broilers. *Biol. Trace Elem. Res.*, 104: 19-31.
35. Okeke, R.O., I.I. Ujah, P.A.C. Okoye, V.I.E. Ajiwe and C.P. Eze, 2015. Effect of different levels of cadmium, lead and arsenic on the growth performance of broiler and layer chickens. *J. Applied Chem.*, 8: 57-59.
36. Bokori, J., S. Fekete, I. Kadar, J. Koncz, F. Vetesi and M. Albert, 1995. Complex study of the physiological role of cadmium. III. Cadmium loading trials on broiler chickens. *Acta Vet. Hung.*, 43: 195-228.
37. Bokori, J., S. Fekete, R. Glavit, I. Kaday, J. Konez and L. Kovari, 1996. Complex study of the physiological role of cadmium. IV. Effects of prolonged dietary exposure of broiler chickens to cadmium. *Acta Vet. Hung.*, 44: 57-74.
38. Abduljaleel, S.A. and M. Shuhaimi-Othman, 2013. Toxicity of cadmium and lead in *Gallus gallus domesticus* assessment of body weight and metal content in tissues after metal dietary supplements. *Pak. J. Biol. Sci.*, 16: 1551-1556.