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EFFECT OF ZINC AND EDTA ON LEAD AND CADMIUM RESIDUES IN BUFFALO CALVES

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SUMMARY

This study was conducted to investigate the ameliorative effect of dietary supplementation of either Zn or EDTA or zinc+EDTA on reducing plasma and tissue residues using 20 buffalo calves' naturally exposed to environmental lead (Pb) and cadmium (Cd) reared in Mahlet-Mosa Farm in Kafr El–Shiekh governorate. Lead and cadmium levels were quantified fortnightly in blood samples throughout the fattening period (6 month) and the residues in liver, kidney and eye muscles after slaughtering were measured. It was found that the additions of Zn, EDTA and zinc+EDTA reduced the level of Pb in blood by 37, 42 and 20%, respectively; the corresponding residues of cadmium in plasma were 37.5, 50 and 25%, respectively compared to the control group. Also, the treatments effectively reduced (P<0.05) the lead and cadmium residues in soft tissues (liver, kidney and eye muscles). The levels of lead in liver were lower (P<0.05) in EDTA, zinc and zinc+EDTA treated groups (0.27, 0.20 and 0.31ppm), than the control group 0.55ppm. Meanwhile, the residues of lead in kidney were varied (P<0.05) among the different treatments being 0.17, 0.21 and 0.45ppm in EDTA, zinc and EDTA+zinc treated groups, receptively, compared to 0.75ppm in the control group. The residues of lead in the eye muscle were 0.08, 0.06 and 0.11ppm for zinc, EDTA and zinc+EDTA groups, respectively, compared to control group (0.15ppm). The cadmium residues as ppm in liver were less in EDTA (0.18), zinc (023), zinc+EDTA (0.24) treated groups compared to the control group (0.41). In kidney cadmium residues were ranged between 0.27 to 0.36 ppm in all treated groups. The levels of cadmium in the eye muscles were lower (P<0.05) in zinc, EDTA and zinc+EDTA (0.02, 0.03 and 0.03ppm, respectively) than the control group (0.05ppm)

These results showed that dietary addition of zinc, EDTA and zinc+EDTA has the ability to reduce Pb and Cd residues in blood and soft tissue of buffalo calves without any negative effects. Moreover, EDTA addtions had more ameliorative Pb and Cd residues the effects of dietary supplementation of either zinc or zinc+EDTA as insured by blood parameters (Hb, PCV, creatinine, urea, AST and ALT) and soft tissues (liver, kidney and eye muscles Longissimus Lumbarium).

Keywords: Buffalo calves, Pb, Cd, Zn, EDTA supplementation

INTRODUCTION

The diverse deleterious health effect upon exposure to toxic heavy metals in the environment is a matter of serious concern and a global issue. Lead and cadmium are the two most abundant toxic heavy metals in the environment. The common sources of lead and cadmium in nature are diverse including natural and anthropogenic processes such as combustion of coal and mineral oil, smelters, mining and alloy processing units, paint industries, and so forth. (Phillips *et al*., 2003 and Patra *et al*., 2007). The quantity of lead used in the present decade far exceeds the total amount consumed in all previous eras. Cadmium is an important environmental pollutant present in soil, water, air and food. Anthropogenic sources add 3–10 times more cadmium to the atmosphere than natural sources (Patra *et al*., 2011). The build-up of heavy metals in the body can lead to a variety of health problems (Ruff *et al*., 1996) associated with pathologies in the nervous, cardiovascular, hematopoietic, gastrointestinal and immunological systems as well as renal dysfunction, anemia, liver problems. Cadmium has received much attention because of its reported toxicity to humans. It is highly toxic element that accumulates in biological systems. Its toxicity is manifested by kidney dysfunction, hypertension, hepatic injury, reproductive toxicity, lung damage, arteriosclerosis, growth inhibition, damage to the nervous system, bone demineralization and endocrine disruption (Kim, 2004 and Gonzalez-Willer *et al*., 2006)).

Many researchers have been investigated that the use of certain chelating and antioxidant agents can reduce the accumulation of the heavy metals in the body. Mikirova *et al*. (2011) stated that, chelating therapy has been proposed for removing poisonous metals such as Pb, Hg, Cd, and Al, as well as reducing abnormal accumulations of trace nutrients such as Fe, Cu, and Zn. Chelating therapy employs anionic chelating agents such as ethylene diamine tetra acetic acid (EDTA) to bind heavy metal cautions found in the blood. Once EDTA is bound, these metals can be removed through the kidney. Also, the uses of antioxidants such as zinc (Zn) can be useful in reducing heavy metals accumulation. Kumar *et al.* (2012) mentioned that increased dietary intake of zinc reduces the accumulation and toxicity of lead, probably by decreasing its intestinal absorption. Also, Zn and Pb compete for similar binding sites on a metallothionin-like transport protein, and that the presence of Zn reduces absorption of Pb from the gastrointestinal tract (Flora and Tandon, 1990). Supplementation of Zn in the diet of heavy metals exposed animals can help to reduce the adverse effect of lead and improve the blood lymphocyte population, phagocytosis and killing ability by macrophages. Animals deficient in zinc are more susceptible to be poisoned with lead, because there is increased absorption of this mineral element (Alonso *et al.,* 2004)

This investigation was to determine the effect of Zn and EDTA, addition on some blood parameters and soft tissues of buffalo's calves exposed naturally to the lead and cadmium in Mhalet Mosa farm.

MATERLIAS And METHODS

The current experiment was conducted in Mehalet–Mousa Farm, Kafr El–Shiekh governorate, which belongs to Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamations (MALR), Dokki, Giza, Egypt. The farm is located in the northern part of the Nile Delta, about 131 km to the north of Cairo and about 1.5 km away from the main road. There was a train station nearby the farm about 200-250 m. and bus station was far from the farm about 1.5 Km.

The experiment started in Mars, and contained till Sep 2013 (180 days). A total of 20 male buffalo calves (*Bublaus bublis*) were used, ranged between 8-12 months of age and the average of initial live body weight of the animals was 194±5.0 kg (the animals were weighed fortnightly) and received the same feeding system, feeding was according to NRC allowances for fattening (NRC, 2005). Animals were housed at shaded open yards. Drinking water was from underground water and offered *ad lib*itum twice daily. The animals were divided into 4 equal groups (5 animals each), the first group, T1 control group was fed the basal diet without any additions. (The basal diet contains concentrate feed mixture and rice straw). The second group, T2 was fed the basal diet + 1g zinc (Several zinc) /calf/day. Several zinc, is produced by Egyptian United Company (EUC), Egypt. The ingredients of this compound are; 10 million IU/kg Vit. A, 20 million IU/kg Vit. D3, 10000 mg/kg Vit A and 50000 mg/kg zinc bacitracin. The ingredients in the product were carried by calcium carbonate up to1 kg. The third group, T3 was fed the basal diet which supplemented with 1g EDTA /calf/day, The fourth group, T4 was fed the basal diet + 1g zinc + 1g EDTA /calf/day, the EDTA is produced by ADWIC United Company (El-Nasr Pharmaceutical Chemical), Egypt.

A total of 150 ml of water and 250g of feed samples were collected monthly during the experimental period for further analysis and quantify its lead and cadmium contains according to AOAC (1990). Blood samples (10ml) were collected fortnightly from the experimental animals from the jugular vein from each animal and collected in heparinized tubes to determine the residues of Pb and Cd in cell component (the residue of blood after centrifuging the sample) by using Atomic Absorption Spectrophotometer at wave length of 217 nm according to the method described by AOAC (1990). The plasma was used to determine some hematological and biochemical blood parameters to test both liver and kidney functions. Quantitative colorimetric determination of hemoglobin (Hb, g/dl) (Eilers, 1967), creatinine (mg/dl) (Husdan and Report, 1968), testing kits of both Hb and creatinine produced by Egyptian American Company for Laboratory Services, Bio-Diagnostic Dokki-Giza Egypt, the absorbance readings were at 540, 495, respectively. Testing of AST and ALT (u/l) was executed by using Kits of SENTINAL CH., 20155 Milan, Italy. The absorbance reading was 546 nm, and the linearity for creatinine is 10 mg/dl for both serum and plasma. Packed cell volume (PCV, %) (Dacie and Lewis, 1984) was obtained by applying a capillary column of 100 mm heparinzed blood in micro-hematocrit tubule, centrifuged for 15 minutes at 12000 rpm, and measured against a scale as percentage of the whole blood column.

At the end of experiment, three animals from each group (n=12 calves) were chosen randomly to be slaughtered to collect their tissues (liver, kidney and eye muscle *(Longissimus lumbarium* at 9, 10, 11 ribs). The residual content of lead and cadmium in these tissues and chemical analysis of eye muscles was determined according to AOAC (1990).

The data were analyzed using the general linear Model procedure of SAS (2000), test the efficiency of the amelioration of lead and cadmium or both from the blood, the following statistical model was applied to the data set:

$Y_{ij} = \mu + T_i + e_{ij}$

Y_{ij} =Experimental observation

μ =overall mean.

T_i = Effect of treatment (I=1-4) 1= control (no addition), 2= zinc Bacitracin addition and 3= EDTA addition and 4= zinc+ EDTA addition.

e_{ij} = Experimental error assumed to be randomly distributed (0, σ2)

RESULTS AND DISCUSSION

Lead and cadmium in water and feed:

The present data showed that lead and cadmium concentration in drinking water (table1), in Mahalet-Mousa farm were 1.62 and 0.31ppm, respectively. The results were higher than the primate limits for lead and cadmium (0.05 and 0.001ppm), respectively), according to WHO (1984). This may be related to the farm's place which is near to railway station, the main road and within the human buildings.

Table 1. Lead and cadmium residues in water and feeds calves (LSM±SE)

Item	Drinking water (ppm)	Feed (mg/kg DM)
Lead	1.62 ±0.236	0.89 ±0.145
Cadmium	0.31 ±0.056	0.35 ±0.057

The present data were lower than that obtained by Abdel-Rahman, Samah (2005*)* and Hamza (2005*).* They found that drinking water lead in Mahlet Mosa farm was 1.78 and 2.0ppm, and cadmium was 0.22ppm. El-Kholy (2007), found that the concentration of lead, and cadmium in drinking water in Mehalet-Mousa farm were 2.0 and 0.22ppm, respectively. Abdel-Nasser *et al.* (1996*)* reported that the lead concentration in twenty five samples of water from Upper Egypt Rive Nile ranged between 0.79 and 1.94 ppm and cadmium concentration ranged between 0.007 and 0.0015ppm. Mohamed *et al* (1998) found that the lead and cadmium level's in raw water Nile River sampling were 8.74 and 0.05mg/l respectively, and 0.88 and 0.63 mg/l in drinking water, respectively.

Present results show that the means of lead and cadmium concentration in feed were 0.89 and 0.35mg/kg DM, respectively, which was higher than that obtained by EOSQC (2010), and WHO (1984). They stated that the permissible limits of lead and cadmium in feed were (0.5 and 0.05 mg/kg DM), respectively. The present data were higher than that reported by El-Kholy (2005) and Hamza (2005), who found that the lead and cadmium residue in feed collected from the same farm were 0.73 and 0.31 mg/kg DM, respectively. While, Khalaf and Abdel-Aal (1999), recorded that lead concentration in vegetation growing in polluted area in Helwan was 183ppm. Also, Zip (2003), found that the mean concentration of lead in feed was 4.75 mg/kg DM. The risk of exposure of the animals to the contaminants may be attributed to grazing and drink water from ditches, streams, rivers and other possibilities which contaminated water sources. Gehad *et al.,* (2009)

Effect of treatment on lead and cadmium residues in blood plasma:

Lead and cadmium residues in blood are shown in table (2). The over all mean of lead in plasma were 0.65, 0.41, 0.38 and 0.52 ppm for control, zinc, EDTA and zinc+EDTA groups, respectively. The dietary addition of zinc, EDTA and zinc+EDTA reduced ($p<0.05$) lead in blood plasma by 37, 42 and 20% than the control groups, respectively. The present data are in agreement with Kumer *et al.* (2012) who stated that the amount of Zn addition in the diet contaminated with Pb may be more effective to counteract the adverse effect of Pb in goat kids. This could be attributed to, that Zn and Pb compete for similar binding sites on a metallothionin like transport protein, and that the presence of Zn reduces absorption of Pb from the gastrointestinal tract (Flora and Tandon, 1990). Patra *et al.* (2011) mentioned that Zn addition competes and effectively reduces the availability of binding sites for lead uptake. So, supplementation of Zn in the diet of heavy metals exposed animals can help to reduce the adverse effect of lead. Also, the clearance effect of EDTA on lead through increasing urinary lead level thus reduces blood lead. The present data are in agreement with*,* Yasir *et al.* (2008) and Patra *et al*. (2010) who mentioned the beneficial effects of using EDTA in reducing blood lead. (Zhang *et al*., 2005). Lead is not involved in any physiological functions.

Table 2. Lead and cadmium residues (ppm) in blood plasma of buffalo calves as affected by different treatments (LSM±SE)

Treatment	Lead (ppm)	Cadmium (ppm)
Control	0.646 [a] ±0.074	0.083 [a] ±0.008
Zinc	0.413 [bc] ±0.055	0.051 [bc] ±0.012
EDTA	0.383 [c] ±0.040	0.040 [c] ±0.062
EDTA+Zinc	0.520 [ab] ±0.092	0.060 [b] ±0.006

L.S. Means within the same column having different superscript letters differ ($P<0.05$)

The over all means of cadmium residues in blood plasma were 0.08, 0.05, 0.04 and 0.06ppm for control, zinc, EDTA and Zinc+EDTA groups respectively, the dietary addition reduced cadmium blood plasma which were lower than control group by 37.5, 50 and 25%, respectively.

To explain the role of Zn in reducing the Cd level in blood, the amount of absorbed Cd through gastrointestinal tract is relatively lower than divalent Zn and Fe cautions, so the deficiencies of these cautions leads to increase the bioavailability of Cd, once absorbed, Cd circulates in red blood cells and bound to albumin (Reeves and Chaney, 2001, 2002, and 2004). Concerning the effect of EDTA, it has the ability of increasing the urinary Cd excretion (Waters *et al*., 2001).

The present data are higher than that reported by Todd *et al.* (1995), who found that lead poisoning level in adult cattle blood was 0.35ppm. Mirand *et al* (2005) found that cadmium level in blood was 0.403 mg/dl. Lopez *et al.* (2000) found that cadmium concentration in blood calves and cows were 0.373 and 0.449 mg/dl respectively,

Effect of treatment on some hematological and biochemical blood parameters:

The present data of the relevant blood hematological and biochemical parameters were within the normal physiological range which confirms the safety of using zinc and EDTA in rations, and there is no negative impact on animal health.

The present study shows a significant decrease in hemoglobin values of the control calves (Hb g/dl) (table 2) than zinc, EDTA and zinc+EDTA treatments and they were (10.28, 15.17, 16.33 and 12.91 g/dl), respectively. These reductions could be attributed to the high level of lead in control blood and its effect on hemoglobin synthesis. Neathry and Miller (1975) mentioned that lead inhibits the utilization of iron and biosynthesis of heme and causes anemia. Wayne *et al* (1995) mentioned that lead impairs the formation of red blood cells and Kwong *et al* (2004) found that lead had a higher affinity for the side of five amino acids, trancferrin, mucine, mobilferin, DMT and hemoglobin enzymes of heme synthesis which might explain why lead has been found to bind the numerous protein crucial for uptake, transport and utilization of iron.

The values of packed cell volume (PCV) ranged between 37.28 and 40.78% (Table 2). There were significant difference ($P<0.05$) among control, EDTA+zinc and zinc, EDTA groups, and the highest values were in EDTA group and the lowest for the control group. The present data are in agreement with that reported by El-Kholy (2005) who found that the Hb concentration and PVC values for buffalo calves in Mahalet- mosa farm ranged between 8.84 g/dl to 40.93%, respectively. Telisman *et al* (1990), found no decrease in PCV in cattle reared in the vicinity of lead smelter.

The values of creatinine in blood were (1.43, 1.16, 1.09 and 1.25g/dl) for control, zinc, EDTA and zinc+EDTA groups, respectively. The difference among the groups was significant ($P<0.05$), and the data were within the normal physiological mean (1-2 g/dl) which was mentioned by Reece (1991).

Results indicate that adding zinc or EDTA does not affect the kidney function in eliminating creatinine outside the body.

Urea concentration was within the normal physiological range in zinc and EDTA treatments; but the control group was higher ($P<0.05$) than the other groups, the values were (36.14, 30.05, 28.22 and 31.59 mg/dl) for control, zinc, EDTA and zinc+EDTA animals, respectively. The results agree with the findings by Walid (1997), Swarup and Dwiredi (1992), and El-Massarawy (1997), who recorded a significant increase in creatinine and urea in blood due to lead pollution. Gamal *et al.* (2009). found that EDTA administration counteracts all changes in kidney function appears more or less like the normal group. Gouda *et al.* (1986), stated that there was a significant increase in creatinine concentration in goats received 10 mg lead acetate/kg body weight for 24 weeks. They also recorded a significant increase in creatinine concentration in male rabbits treated with 100 mg/l lead acetate/kg body weight for 36 weeks.

Total protein, albumin, globulin and A/G, were in the normal physiological ranges reported by Reece (1991) in zinc, EDTA and zinc+EDTA treatments; but zinc, EDTA and zinc+ EDTA treatments in total protein was higher significantly ($P<0.05$), than the control group by 22.2, 9 and 4% , respectively, and these findings agree with that reported by Walid (1997).

The overall mean of AST activity (Table 3) was 33.77, 21.15, 18.75 and 29.22 u/l for control, zinc, EDTA and zinc+EDTA treatments, respectively. There was a significant difference ($P<0.05$) among all groups in AST concentration. These values were within the normal physiological range according to Bernard (1948), who stated that the normal range of AST in cows is 8-50 u/l.

Concerning liver enzymes, the overall mean of ALT activity (Table, 3) ranged between 40.55 to 21.48u/l. The data showed that there were significant differences ($P<0.05$) among all treatments and the highest in the control and, the lowest in EDTA treatment. In ruminants, ALT levels in liver are lower than the AST levels (Doxy, 1971). So when liver is exposed to toxic agent that will result in high serum AST level and slight rise in ALT level. The results agree with that reported by Shalaby (2007), who stated that EDTA addition improved AST and ALT activity. El-Massarawy (1997), found a decrease in ALT and AST in male albino rats received 5 mg lead acetate/liter distilled water.

Effect of adding Zn and EDTA on Lead and cadmium residues in soft tissues:

The residues of lead and cadmium in soft tissues (liver, kidney and eye muscle, *Longissimus lumbarium*) are presented in Table (4). The results showed the obvious differences between control and treated groups in Pb and Cd residues in tissues. This indicating the effectiveness of both treatments (Zn and EDTA) in reducing Pb and Cd residues in different tissues than the control group, and the lowest recorded level of both Pb and Cd was in eye muscle in comparison to liver and kidney. The main site of heavy metals accumulation is liver. (Alonso *et al*., (2003), mentioned that muscles have the lowest capacity to accumulate lead). Our results are in agreement with the findings by Allama and Elhammaly (2009), Szakova *et al.* (2009) and Imed *et al.* (2010). They reported that the dietary supplementation of EDTA or zinc had an ameliorative effect on cadmium toxicity.

The present results show that, the means of lead residues in buffalo calves liver were significantly lower ($P<0.05$) in, zinc, EDTA and zinc+ EDTA groups than the control, (0.27, 0.20 and 0.31 vs. 0.55, ppm), respectively, which were within the permissible limit (2 ppm) recommended by EOSQC (2010). The lowest lead residues among the experimental groups were noticed with in EDTA group followed by the zinc group. On the other hand, the control group was higher than the standard level (0.5ppm), while zinc, EDTA and zinc+EDTA, treatments showed that lead residues in liver were safe and less than standard level. Todd *et al*. (1995) found that the highest concentration of lead was found in liver.

Table 3. Some hematological and biochemical parameters in blood of buffalo calves as affected by different treatments (LSM±SE)

Treatment	Control	Zinc	EDTA	EDTA+Zinc
Hematological parameters				
Hb (g/dl)	$10.28^{d} \pm 0.761$	$15.17^{b} \pm 0.418$	$16.33^{a} \pm 0.648$	$12.91^{b} \pm 0.502$
PCV (%)	$37.67^{c} \pm 0.642$	$39.11^{b} \pm 0.622$	$40.78^{a} \pm 0.469$	$37.28^{c} \pm 0.743$
		Biochemical parameters		
Creatinine, mg/dl	$1.43^{a} \pm 0.168$	$1.16^{a} \pm 0.190$	$1.09^{a} \pm 0.132$	$1.25^{a} \pm 0.212$
Urea, mg/dl	$36.14^{a} \pm 0.521$	$30.05^{cb} \pm 0.828$	$28.22^{c} \pm 1.780$	$31.59^{b} \pm 1.390$
Total protein, g/dl	$6.27^{b} \pm 0.149$	$7.66^{a} \pm 0.176$	$6.89^{b} \pm 0.160$	$6.56^{b} \pm 0.455$
albumin, g/dl	$3.86^{b} \pm 0.105$	$4.61^{a} \pm 0.199$	$4.13^{ab} \pm 0.342$	$4.08^{b} \pm 0.314$
globulin, g/dl	$2.41^{a} \pm 0.203$	$3.05^{a} \pm 0.312$	$2.76^{a} \pm 0.249$	$2.48^{a} \pm 0.278$
A/G ratio	$1.61^{a} \pm 0.172$	$1.52^{a} \pm 0.229$	$1.52^{a} \pm 0.259$	$1.65^{a} \pm 0.167$
AST U/L	$33.77^{a} \pm 0.819$	$21.15^{c} \pm 0.838$	$18.52^{d} \pm 0.799$	$29.22^{b} \pm 1.234$
ALT U/L	$40.55^{a} \pm 0.154$	$24.67^{c} \pm 0.837$	$21.48^{d} \pm 0.656$	$31.21^{b} \pm 0.457$

L.S. Means within the same raw having different superscript letters differ (P<0.05)

Table 3. Lead and cadmium residues (ppm) in liver, kidney and eye muscles of buffalo calves as affected by different treatments (LSM±SE)

Treatment	Control	Zinc	EDTA	Zinc+EDTA
		Lead		
Liver	$0.55^{a} \pm 0.087$	$0.27^{c} \pm 0.052$	$0.20c^{bc} \pm 0.017$	$0.31^{b} \pm 0.026$
Kidney	$0.72^{a} \pm 0.96$	$0.23^{c} \pm 0.152$	$0.17^{c} \pm 0.026$	$0.45^{b} \pm 0.066$
Eye muscle	$0.15^{a} \pm 0.017$	$0.08^{bc} \pm 0.020$	$0.06^{c} \pm 0.020$	$0.11^{b} \pm 0.020$
		Cadmium		
Liver	$0.41^{a} \pm 0.036$	$0.23^{b} \pm 0.026$	$0.18^{c} \pm 0.027$	$0.24^{b} \pm 0.030$
Kidney	$0.36^{a} \pm 0.036$	$0.29^{b} \pm 0.064$	$0.27^{b} \pm 0.027$	$0.30^{b} \pm 0.036$
Eye muscle	$0.05^{a} \pm 0.010$	$002^{b} \pm 0.003$	$0.03^{b} \pm 0.002$	$0.03^{b} \pm 0.004$

L.S. Means within the same raw having different superscript letters differ (P<0.05).

The means lead residue in kidney were 0.72, 0.23, 0.17 and 0.45ppm in control, zinc, EDTA and zinc+EDTA groups, respectively. Lead in zinc and EDTA groups were lower (P<0.05) than that in control and zinc+EDTA groups, the residues of lead in kidneys of the groups having an addition of either zinc or EDTA and zinc+EDTA groups were lower by 71, 76 and 38%, respectively than the control. The results are lower than that found by Gehad *et al*., 2009) . They found that the mean values of cadmium residue in liver and kidney samples of cow were 1.017 and 1.128ppm respectively, when collected one hundred samples liver and kidney from different Egyptian markets. Shaaban *et al*. (1992) found that the highest level of lead residues in kidney was 4.914ppm in group aged 6-12 months. Lead accumulates in liver and kidney because liver is an important storage, redistribution, transformation and detoxification organ and kidney is a key waste of metabolism, water and electrolyte balance and acid-base concentration regulation organ. The level of cadmium residue in kidney was higher than that in liver Gehad *et al.,* (2009) and Iwegbue *et al*., (2008) The higher concentration of cadmium in the kidney tissue is due to detoxification of the organ where these metals are accumulated (Falandysz, 1994).

The means lead residues in eye muscle (*Longissimus lumbarium*) were 0.15, 0.08, 0.06 and 0.11ppm in control, zinc, EDTA and zinc+EDTA treatments, respectively. The residues of lead in eye muscle after addition of zinc, EDTA or zinc+EDTA were sufficiently lower (P<0.05) by 46, 60 and 26%, respectively than the control. Allama and Elhammaly (2009), Todd *et al*. (1995) and Dolye and Spaulding (1987), found that adding EDTA reduce residues of lead liver, kidney and muscle than the control and concluded that EDTA may be useful for the treatment of lead toxicity. Also, zinc can reduce lead residues in tissues through reducing its absorption from gastrointestinal tract, thus reducing its accumulation (Gill *et al.,* 2011); while EDTA reduces Pb lead through increases its urinary excretion (Mikirora *et al.,* 2011). The results agree with Gamal *et al.* (2009), who found that ration supplemented with EDTA may be useful in the protection of tissues from lead toxicity. Daoud *et al*. (1998) found that the residues of lead in cattle liver reared in El-Sharkia and Zagazig government, Egypt ranged from 0.7 to 1.156 ppm and in kidney 1.28ppm, while the lowest lead concentration residues ranged from 0.555 to 1.303 in eye muscles slaughtered cattle. However, Farmer and Farmer (2000) found that lead was concentrated in kidney (97.0-170.5 ppm) than the liver (27.3-97.4 ppm). These data are lower than reported by Abdel-Rhman, Samah (2005) who found lead residues in buffalo calves in Mahlet Mosa farm were 4.0, 2.6 and 0.9 ppm, in liver, kidney and eye muscle, respectively. Also, Hugh *et al.* (1990) mentioned that lead residues in muscles were lower than that in liver and kidney.

Table (4) shows that the dietary addition of Zn, EDTA and zinc+EDTA reduced cadmium residual' levels than the control treatment in liver, kidney and eye muscle, respectively. The mean cadmium residues in buffalo calves liver ranged between 0.41 and 0.18 ppm being lower ($P<0.05$) in EDTA, zinc and zinc+EDTA groups than the control. Our data agree with Abo-Arab (2001) and Lopez *et al.* (2003) and Szakova *et al*. (2009). Dietary supplementation of EDTA and zinc had an ameliorative effect on the toxicity of cadmium in liver

The overall mean of cadmium residues of buffalo calves kidney were 0.36, 0.29, 0.27and 0.30ppm in control, zinc,EDTA and zinc+EDTA groups, respectively. The residue of cadmium in EDTA group was the lowest and the control group was the highest. Effectiveness of the addition of zinc on reducing Cd residues could be due to, that zinc has a role in inducing renal metallotionein and protects the kidney from dissolved Cd/metallotionein when Cd presents in liver, metallothionein makes a complex thus reducing the amount of free Cd that injury hypatocyte, this complex is dissolved and reabsorbed in the proximal tubules (in kidney) producing free Cd which can again be bound to newly synthesized metallotionein, thus protecting the kidney from the damage of cellular membrane of the renal tubules. (Patric, 2003). While, EDTA reducing level of Cd as same as Pb by increase the Cd urinary excretion (Waters *et al*., 2001). Also, EDTA has the ability in reducing the general metal toxicity (Blanusa *et al.,* 2005 and Anderson, 1984). The present data are lower than reported by Gehad *et al.* (2009) who found that Cd residues were 1.017 and 1.128 ppm in liver and kidney, respectively.

The mean range of cadmium residues in eye muscle (*(Longissimus lumbarium*) in the different groups were 0.05 to 0.02 ppm. The cadmium residues in eye muscle of buffalo calves were higher in control group than the other groups. These results agree with those of Dolye and Spaulding (1987). They found that the normal cadmium values in muscles were lower than that found in liver and kidney, Gamal *et al.* (2009) found that EDTA supplement may be useful in reducing cadmium toxicity from tissues. Nwude *et al.* (2010) they found Cd in cow tissues was 0.532 ppm.

CONCLUSION

The present study declared the possible amelioration of the residues of lead and cadmium in buffalo calves using zinc, EDTA and zinc+EDTA as a dietary addition. Adding zinc with EDTA decreased the amelioration effect on the residues of lead and cadmium in blood and animal's soft tissues in compare with addition of either zinc or EDTA alone. The dietary supplementation of EDTA was the most efficient in reducing lead and cadmium residues in blood, liver, kidney and eye muscle among all treatments.

Screening of the residues of lead and cadmium in buffalo showed that the eye muscle had the lowest residues of these heavy metals.

REFERENCES

Abdel-Nasser, M. A.A. Shaaban, Seham, M. Aly and Manal, M. Sayed. 1996. Lead, copper, mercury and cadmium levels in Rive Nile waters at some Assiut egions, Egypt. Assiut. Vet. Med., J. 34: 85-93.

Abdel-Rahman, Samah, M. 2005. Study on Lead Level in blood and muscles of Buffalo in Two Different Farms. M.Sc. Thesis, Fac. Agric, Cairo Univ., Egypt, 122p.

Abou-Arab, A.A.K. 2001. Heavy metal content in Egyptia meat and the role of detergent washing on their levels. Food Chem. Toxical. 3: 593-599.

Allama, G.H.N. and Elhammaly, H.S. 2009. Melatonin EDTA ameliorates cadmium induced oxidative damage and morphological changes in kidney of rat. The open neuron- Endo. J. 2: 1-9.

Alonso, L.M., Miranda, M., Castillo, C., Hernandez Alonso, L.M., Miranda, M., Castillo, C., Hernandez J., Benedito, J.L. and Montana F.P., 2003. Cadmium and lead accumulation in cattle in North West Spain. Vet. Human Toxic. 45(3): 128-192.

Alonso, M.L., Mantaña, F.P., Miranda, M., Castilho, C., Hernández, J., Benedito, J.L. 2004. Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. Biometals. 17, 397-398.

Andersen, O., 1984. "Chelating of cadmium," *Environmental Health Perspectives*, vol. 54, pp. 249–266.

AOAC, 1990. Official Methods of Analysis 14^{th} Ed., Association of Official Analytical Chemists, Washington, D. C., USA.

Bernard, L.O. 1948. Hawks Physiological Chemistry. 4th Ed., McGRAW-Hill Book Company. Pp. 977-979.

Blanusa, M., Varnai, V. M., Piasek, M. and Kostial, K. 2005. "Chelators as antidotes of metal toxicity: therapeutic and experimental aspects," Current Medicinal Chemistry, vol. 12, no. 23, pp. 2771–2794.

Dacia, J. V. and Lewis, S. M. 1984. Practical Hematology, sixth edition, ELBS and Churchill Livingston London, UK.

Daoud, J. R., Kamel, A.A. and Moustafa, Abo. B. R. 1998. Determination of lead, cadmium and copper residues in muscles, liver and kidneys of slaughtered cattle and sheep in El-Sharkia government. Vet. Med. J., Giza., 46(4): 339-348.

Dolye, J.J. and Spaulding, J.E. 1987. Toxic and essential trace elements in meat: a Reviow. J. Anim. Sci., 74(2): 398-419.

Doxy, D.L. 1971. "Veterinary Clinical Pathology". Bailliere Tindall, London, UK, p175.

Eilers, R. J. 1967. Notification of final adoption of an international method and standard solution for hemoglobinometry specification for preparation of standared solution. Am. J. Clin. Pathol. 47(2): 212-214.

El-Kholy, A.F. 2007. Studies on Milk Proudaction in Egyptian Buffalo. M. Sc. Thesis, Fac. Agric, Cairo Univ., Egypt, 120p.

El-Kholy, A.F., Abd-el Rahman, S., Hafez, Y.M. and Hassan, H.Z. 2005. Some hematological and biochemical parameters and lead residues in blood and tissues throughout fattening of male buffaloes in two different farms. Egypt. J. Basic Appl. Physiol., 4(1): 221-231.

El-Massarawy, A.M.D. 1997. Studies in the effect of some heavy metals on testis, thyroid, liver and kidney function in albino rats. PhD. Thesis, Fac. Vet. Med., Cairo University.

EOSQC, 2010. Maximum for heavy metal contamination in food, Egyptian Organization of Standardization and Quality Control, Es 6636.

Farmer, A.A. and Farmer, M.A, 2000. Concentrations of cadmium lead and zinc in livestock feed and oranges around a metal proudaction center in eastern Kazakhstan. J. Sci. of the total Environ. 257: 53-60.

Flora, S. J, and Tandon, S. K, 1990. Beneficial effects of zinc supplementation during chelation treatment of lead intoxication in rats. Toxically. 64, 129 – 39.

Gamal, H., Allama, N., and Elhammaly, H. S. 2009. Melatonin and EDTA ameliorates cadmium induced oxidative damage and morphological changes in the kidney of rat. The open Neuron-Endocrinol. J., 2: 1–9.

Gehad, F.A., Fath, El–Bab and Thabet, H. S.M. 2009. Heavy metal residues in some edible offal's of cow and their health hazards. J. Egypt Med. Asocial., 69:149 – 156.

Gil, H., Kang, W. E. J., Lee, K. H., Yang, J. O., Lee, E. Y., and Hong, S. Y. 2011. "Effect of glutathione on the cadmium chelation of EDTA in a patient with cadmium intoxication," *Human and Experimental Toxicology*, vol. 30, no. 1, pp., 79–83.

Gouda, I.M., Aziz, S. A. A., Ahmed, A. A., Lotti, M.M. and Soliman, M. M. 1986. Changes in some kidney functions in experimentally poisoned goats. Arch. Exp. Vet. Med.,40:242-249.

Hamza, R. A. 2005. Studies on Milk Production in Egyptian Buffalo. M.Sc. Thesis, Fac. Agric, Cairo Univ., Egypt, p120.

Hugh, D., Riordan, M.D., Emanual, C.M.D. and Marvain Dirks, B.D. 1990. Mineral excretion associated with EDTA chelating therapy. J. Advan. Med., 3: 111-123.

Husdan, H. and Report, A. 1968. Estimation of creatinine by Jaffe reaction. A comarision methods., Clinical Chemistry, 14: 222-238.

Imed, M., Hmmouda, F., Jihene, H., Tarek, B., Khled, S and Abd El Hamid, K. 2010. Reversal of cadmium induced oxidative stress in rat erythrocytes by selenium, zinc or their combination. Exp. Toxicol. Pathol., ogy 62:218-288.

Khalaf-Allah, S.S. and Abdel-Aal, S.A. 1999. Effect of lead emission on sheep grazing in heavy industrized area in Helwan, Egypt. Assiut Vet. Med. J., 40(80):147-155

Kumara, M., H. Kaura, B.T. Phondbab, G. Chandrac, R.S. Dekaa, V. Sharma. 2012. Ameliorative effect of zinc supplementation to lead exposed goat kids on immune status. Scientific J. Anim. Sci. 1(5) 154-158.

Kumara, M., H. Kaura, B.T. Phondbab, G. Chandrac, R.S. Dekaa, V. Sharma. 2012. Ameliorative effect of zinc supplementation to lead exposed goat kids on immune status. Scientific J. Anim. Sci. 1(5) 154-158.

Kwong, T. W., Friello, P. and Semba, D.R. 2004. Interactions between iron deficiency and lead poisoning epidemiology and pathogenesis J. Sci. of the total Environ., 330: 21-37.

Lopez, A. M., Prieto, M.F., Miranda, M. and Hernandez, J., 2003. Cadmium and lead accumulation in cattle in North West Spain. Vet. Human Toxicol., 45: 128-130.

Lopez, A.M., Nemedito, J.L., Miranda M., Castillo, Hernanth 1dez, J. and Shore, R.F., 2000. Toxic and trace elements in liver, kidney and meat from cattle slaughtered in Galicia North West Spain. Food Addit. Contam., 17: 447-457.

Mikirova, N., Joseph C. and Ronald. 2011. EDTA chelating therapy in the treatment of toxic metals exposure. *Spatula DD. 2011; 1(2):81-89*.

Miranda, M., Alonso, M.L., Castillo, C., Hernandez, J. and Benedito, J.L., 2005. Effect of moderate pollution on toxic and trace metal levels in calves from polluted area of northern Spain Environ. Int. J., 31(4):543-548 (Abstr.).

Mohamed, Y.S.; Mira, I.R.A. and El-Khawask, M., 2008. Led levels in tissues of animals slaughtered in Ismailia slaughterhouse. Vet. Med. J. Giza, 56:21-28.

Neathry, M.W. and Miller, W.J. 1975. Metabolism and toxicity of cadmium, mercury and lead in animals. A Review. J. Dairy Sci., 58: 1767-1779.

NRC, 2005. Mineral Tolerances of Animals, National Academic Press, Washington DC, USA, 157p.

Nwude, D. O., Okoye, .P.A.C. and Babayemi, J. O. 2010. Blood heavy metal levels in cows at slaughter at Awake, abattoir, Nigeria. Int. J. Dairy sci., 5:264-270.

Patra, R. C., Swarup, D. R. and Naresh, D. 2007. "Tail hair as an indicator of environmental exposure of cows to lead and cadmium in different industrial areas," *J. Ecotoxi. Environ. Safety*, Vol. 66 (1): 127–131.

Patra, R. C., Amiya, D., Rautray, K. and Swarup, D. 2011. Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. Veterinary Med., Inter., 1-9.

Patra, R.C., Amiya, K. R. and Swarup, D. 2010. Oxidative stress in lead and cadmium toxicity and its amelioration. Bull. Environ. Contam. Toxicol., 74:1092-1097.

Patric, N.D. 2003. Toxic metals and antioxidants: Part II. The role of antioxidants in arsenic and cadmium toxicity. Alternative Med. Rev., 8 (2): 106-128.

Phillips, C., Gyori, Z. and Kov´acs, B. 2003. "The effect of adding cadmium and lead alone or in combination to the diet of pigs on their growth, carcase composition and reproduction," *J. Sci. Food Agric.,* Vol. 83(13): 1357–1365.

Reece, W.O., 1991. Physiology of Domestic Animals. Lea and Febiger, Philadelphia. USA., 350 P.

Reeves P.G., Chaney R.L. 2004. Marginal nutritional status of zinc, iron, and calcium increases Cd retention in the duodenum and other organs of rats fed rice-based diets Environ Res 96: 311-22.

Reeves PG, Chaney RL. 2002. Nutritional status affects the absorption and whole-body and organ retention of Cd in rats fed rice-based diets. Environ. Sci. Technol.15: 2684-2689.

Reeves, P.G. and Chaney, R. L. 2001. Mineral status of female rats affects the absorption and organ distribution of dietary Cd derived from edible sunflower kernels (Helianthus annulus L.) Environ Res 85: 215-225.

Ruff, H. A., Markowitz, M. E., Bijur, P.E., Rosen, J.F. 1996. Relationships among blood lead levels, iron deficiency, and cognitive development in two-year-old children. Environ Health Percept 104: 180-185.

SAS, 2000. SAS Users Guide. Version 6.12. SAS institute, Cary. NC., USA.

Shaaban, A.A., Shehata, A., Ibrahim, T.A., Seddek, A. SH. And El-Shrief, A.A. 1992. Relationship between certain body electrolytes and lead toxicity in exposed cattle. Assiut Vet. Med. J., 27: 205-222.

Shalaby, A. 2007. Effect of EDTA on toxicity reduction of cadmium in relation to growth, some hematological and biochemical profiles of Nile Tilapa (*Oreochromis niloticus*). J. Fish. Aqua. Sci., 2:100-109.

Swarup, D. and Dwivedi, S. K. 1992. Changes in the blood and cerebrospinal fluid indices in experimental lead toxicity in goats. J. Anim. Sci., 62: 928-931.

Szakova, J., Zidek, V. and Miholova, M. 2009. Influence of elevated content of cadmium and arsenic in containing feeding yeast on organisms of rats. Czech. J. Anim. Sci. 54:1-9.

Telisman, S., Prpic-Majic, D. and Kersang, A. 1990. Relationships between blood lead and indictors of effect in cow's environmental exposed to lead. Toxicology Letters, 52: 347-356.

Todd, M., O'Hara, L.B.C., Pat Mccoy, S.W. and. Cherrill, F. 1995. Lead poisoning and toxic kinetics heifer and fetus treated with CaNa2, EDTA and thiamine. J. Vet. Deign. Invest. 7: 531-537.

Todd, M., Hara, O., Lioyd, B. C., Pat, M. S.W. and Cherrill, F. 1995. Lead poisoning and toxicokinetics in heifer and fetus treated with EDTA and thiamine. J. Vet. Deign. Invest. 7: 531–537.

Walid, S.M.M. 1997. Studies on the effect of lead on reproductive and productive performance of rabbits. Ph D Thesis, Fac. Vet. Med., Cairo University.

Waters, R. S., Bryden, N. A., Patterson, K. Y., Veillon, C., and Anderson, R. A., 2001. "EDTA chelating effects on urinary losses of cadmium, calcium, chromium, cobalt, copper, lead, magnesium, and zinc," *Biol. Trace Element Res.*, Vol. 83 (3): 207–221.

Wayne, A; Landis, G. and Mingo-Ho Yu. 1995. Introduction to Environmental Toxicology. Lewis Publisher, Tokyo, Japan.

WHO, 1984. "Guidelines for Drinking Water Quality" Volume 2, Health Criteria and Other Supporting Information, World Health Organization. pp. 111-119.

Yasir, F.;Muhammad, M.H.;Shoaib, B.A. and Hummad, A.F. 2008. Lead Intoxication: the extent of problem and its management. Pak. J. Physiol., 4:36-41.

Zip, L. 2003. Lead poisoning combined with cadmium in sheep and horses in the vicinity of non-ferrous metal smelters. J. Sci. Total Environ. 309: 117-126.

أثرالزنك والاديتا على متبقيات الرصاص والكادميوم في عجول الجاموس المصري

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تمت اجراء هذه التجربة لقياس تاثير اضافة كلا من الزنك والاديتا أو الاثنين معا على التلوث بالرصاص والكادميوم في دم وبعض الانسجة لعدد 20 عجل جاموسي في مزرعة محلة موسي- معهد بحوث الانتاج الحيواني – محافظة كفر الشيخ.

وقبل بدء التجربة تم عمل دراسة استكشافية لتقدير مستويات التلوث بالرصاص والكادميوم بالمزرعة وشملت الغذاء والماء وكانت النسب اعلى من المعدلات الطبيعية بسبب تواجد المزرعة بالقرب من الطريق الرئيسى ووجود احد محطات السكك الحديدية بالاضافة للتواجد وسط كثافة سكانية مرتفعة ، تم تقسيم الحيونات الى اربعة مجاميع الاولى كونترول بدون اضافات والثانية مضاف اليها 1جم زنك لكل حيوان يوميا والثالثة مضاف اليها 1جم اديتا لكل حيوان يوميا والرابعة مضاف اليها 1جم زنك مع 1جم اديتا لكل حيوان يوميا واستمرت التجربة مدة 6 اشهر تم خلالها تحليل عينات الدم وفى نهاية التجربة تم ذبح ثلاث حيونات من كل مجموعة تم اخذ عينات من الكبد والكلية والعضلة العينية لتحليل نسبة الرصاص والكادميوم بها، وقد وجد ان اضافة الزنك اوالاديتا اوالزنك والاديتا معا ادى لتقليل تركيز الرصاص بالدم بمعدل 37 , 42 , 20 % علي التوالي عن مجموعة الكنترول. بينما انخفض تركيز الكادميوم بنسبة 37.5 , 50 , 25% على التوالى بالمقارنة بالكونترول. وكانت النتائج ايجابية عند تقدير تركيزات الرصاص في الكبد للمجاميع الاربعة فقد بلغت 0.27 , 0.20 , 0.31 , 0.55 جزء فى المليون بالتتابع، وكذلك كانت تركيزات الكادميوم فى كبد المجاميع الاربعة 0.23 , .018 , 0.24 , 0.41 جزء فى المليون على التوالى ، كذلك كان لاستخدام الاديتا والزنك تاثير معنوى على خفض تركيز الرصاص والكادميوم لانسجة الكلية والعضلة العينية. كذلك تاثرت العديد من قياسات الدم نتيجة استخدام الاديتا والزنك بالمقارنة بالكونترول والذى شمل الكرياتنين واليوريا كمقياس لوظائف الكلى والبروتين الكلى والالبيومين والجلوبيولين وALT و AST كمقياس لوظائف الكبد بدون اي تأثير سلبي علي صحة الحيوان، ومن الملاحظ ان اضافة الزنك للاديتا قد قلل من تاثيرهما على تقليل مستويات الرصاص والكادميوم بالانسجة السابقة والدم.

وعلي ذلك يتضح التاثير الإيجابي لإستخدام هذه الإضافات في تقليل متبقيات الملوثات البيئية الرصاص والكادميوم.