Effect of Contamination on Cadmium and Lead Concentration in Blood Plasma and Accumulation in Body Tissues of Rabbits

Gaafar H M A

Animal Production Research Institute, Agricultural Research Center, Dokki, Egypt.
hamedgaafar@gmail.com

Abstract: Fifty growing New Zealand White rabbits (30 males and 20 females) of 8 weeks of age and average body weight of 1091.07±4.93 gm were assigned randomly into 5 similar groups. Rabbits in the control group (G_1) were fed pelleted commercial diet containing 0.28 ppm cadmium (Cd) and 17.60 ppm lead (Pb) on DM basis. Diets of G_2 and G_3 were contaminated with cadmium chloride and lead acetate to provide final levels of 0.5 ppm Cd and 30 ppm Pb on DM basis (maximum tolerable levels of Cd and Pb for domestic animals, NRC 1980) and diets of G_4 and G_5 to provide 0.87 ppm Cd and 58.45 ppm Pb on DM basis (as indicated from Cd and Pb contents of berseem from contaminated areas). Results showed that during the first eight weeks of experiment (9-16 weeks of age), the differences in average daily DM intake among the different groups were not significant (P>0.05). While, average daily DM intake during the interval from 17-26 weeks of age and the overall mean of DM intake were significantly lower (P<0.05) for rabbits fed high levels of Cd and Pb diets (G_4 and G_5, respectively) than those fed low levels of Cd and Pb diets (G_2 and G_3, respectively) and the control group (G_1). Rabbits in G_4 and G_5 revealed significantly (P<0.05) the highest Cd and Pb intake followed by those in G_2 and G_3, while those in G_1 had the lowest Cd and Pb intake and increased with advancing age. Rabbits in G_4 and G_5 revealed significantly (P<0.05) the highest plasma Cd and Pb concentrations followed by those in G_2 and G_3, while those in G_1 had the lowest plasma Cd and Pb concentrations and increased with advancing age. Rabbits in G_4 and G_5 revealed significantly (P<0.05) the highest accumulation of Cd and Pb in liver, kidney and muscle followed by those in G_2 and G_3, while those in G_1 had the lowest Cd and Pb accumulation. The accumulation of Cd and Pb were higher in liver and kidney than muscle.
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1. Introduction

Environmental contamination and exposure to heavy metals is a serious growing problem throughout the world. In today's industrial society, there is no one can not escape from exposure to toxic chemicals and metals. In general, heavy metals are systemic toxins with specific neurotoxic, nephrotoxic, fetotoxic and teratogenic effects. Heavy metals can directly influence behavior by impairing mental and neurological function, influencing neurotransmitter production and utilization, and altering numerous metabolic body processes (Neathery and Miller, 1975; Verma *et al*., 1978; Sharma and Street, 1980 and Mahaffey, 1983).

The most sensitive tissues for cadmium and lead exposure were the kidney and liver (Phillips *et al*., 2003). Understanding the global pattern of contamination in biota is useful in evaluating the health of individual species, population and communities, and in assessing potential risks to humans. Heavy metals can enter the food chain from natural and anthropogenic sources, and once in the body, are distributed among tissues or excreted (Burger *et al*., 2002; Massanyi *et al*., 2003). With each step of the food chain, concentrations increase, resulting in bioamplification, with toxicodynamics differing among spaces. Top-level carnivores or omnivores are often used as bioindicators because they usually have much higher levels of contaminants than those that are lower on the food chain (Baykov *et al*., 2003; Chan *et al*., 2004). The amount of lead and cadmium which distributes in the organs and tissues of the animals depends on the interval of exposure, the quantity ingested; the production and reproduction phase of the animals, as well as their age and breed (Baykov *et al*., 2003). Elements toxicity upon the biological systems of animals is affected by the route and form of ingestion as well as by the interaction between essential and toxic elements. Some metals are essential for life, others have unknown biologic function. Those causing poisonings are the ones, which accumulate in the body through the food chain, water and air (Gotal and Crnic, 2002; Wayland *et al*., 2001). The dates in literatures have not criteria for assessment bioaccumulation or distribution of lead and cadmium in the organs and tissues of the animals.

In Egypt, some agriculture lands in north Delta are irrigated by drain water enriched with sewage sludge. Chemical analysis of fresh berseem samples taken from these lands, showed that the contents of cadmium (Cd) ranged from 0.65 to 0.87 ppm and lead (Pb) ranged from 42.70 to 58.45 ppm on DM basis. These contents were higher than the maximum tolerable levels of these elements for domestic animals being 0.50 and 30.00 ppm on DM basis, respectively as recommended by NRC (1980).

The objective of our study was to determine the effect of cadmium and lead concentration on cadmium and lead intake and its concentrations in blood plasma and some tissues of the rabbits.

2. Materials and methods

The current experiment was carried out at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture.

A total of 50 growing New Zealand White rabbits (30 males and 20 females) of 8 weeks of age and average body weight of 1091.07±4.93 gm were assigned randomly into 5 similar groups with 10 rabbits each (6 males and 4 females) as follows:

G_1 (control): fed pelleted commercial diet containing 0.28 ppm cadmium (Cd) and 17.60 ppm lead (Pb) on DM basis.

G_2: fed pelleted commercial diet contaminated with cadmium chloride (0.36 mg / kg diet) to provide Cd content of 0.5 ppm on DM basis (maximum tolerable level of Cd for domestic animals, NRC 1980).

G_3: fed pelleted commercial diet contaminated with lead acetate (19.47 mg / kg diet) to provide Pb content of 30 ppm on DM basis (maximum tolerable level of Pb for domestic animals, NRC 1980).

G_4: fed pelleted commercial diet contaminated with cadmium chloride (0.96 mg / kg diet) to provide Cd content of 0.87 ppm on DM basis (as indicated from Cd content of berseem from contaminated areas).

G_5: fed pelleted commercial diet contaminated with lead acetate (64.13 mg / kg diet) to provide Pb content of 58.45 ppm on DM basis (as indicated from Pb content of berseem from contaminated areas).

Diets of G_2-G_5 were contaminated with the limited doses more than those present in the control diet by spraying cadmium chloride and lead acetate. The pelleted commercial diet was formulated at Atmida Company to cover the nutrients requirements of rabbits. The ingredients and chemical analysis of pelleted commercial diet are shown in Table (1).

Rabbits were fed ad libitum throughout the experimental period (18 weeks) from June to October 2004 and daily feed intake was recorded. Water was available all the day round from water nipple.

Blood samples were taken biweekly from the jugular vein in dry clean plastic tube containing heparin as anticoagulant. Samples were centrifuged at 4000 rpm for 15 minutes to obtain plasma. At the end of the experiment, three rabbits from each group were chosen randomly, slaughtered after fasting over night. Upon completion of bleeding, animals were skinned, dressed out and samples of liver, kidneys and muscle were taken. The samples of commercial diet, blood plasma, liver, kidneys and muscle were prepared for cadmium and lead determination according to the methods of AOAC (1990). The concentration of cadmium and lead were determined by Atomic Absorption Spectrophotometer (Perkin Elmer 2380).

Data was statistically analyzed using general linear model procedure adapted by SPSS for Windows (2008) for user's guide with one-way ANOVA. Also, Duncan test within SPSS was done to determine the level of significance between means.

3. Results and discussion

Average daily DM intake of growing rabbits in different groups during different intervals of age is presented in Table (2). During the first eight weeks of experiment (9-16 weeks of age), the differences in average daily DM intake among the different groups were not significant ($P>0.05$). While, average daily DM intake during the interval from 17-26 weeks of age and the overall mean of DM intake were significantly lower ($P<0.05$) for rabbits fed high levels of Cd and Pb diets (G4 and G5, respectively) than those fed low levels of Cd and Pb diets (G2 and G3, respectively) and the control group (G1).

It is of interest to note that average daily DM intake showed marked increase with age in contaminated diet groups as in the control group during the interval from 9 to 16 weeks of age. While by advancing age from 17 to 26 weeks, the average daily DM intake of contaminated diet groups was lower than that obtained for the control group, being the lowest in G4 and G5. These results agreed with those obtained by Phillips *et al.* (2005) who found that the ewes were offered a choice of the two herbages and they ate significantly more of the uncontaminated grass and sheep partially avoided herbage with a high Cd concentration.

The intake of cadmium (Cd) by growing rabbits is shown in Table (3). Cadmium intake by rabbits increased significantly ($P<0.05$) with increasing the level of dietary Cd contamination. Which, rabbits in G4 revealed significantly ($P<0.05$) the highest Cd intake followed by those in G2, while those in G1, G3 and G5 had the lowest Cd intake and was nearly similar. Moreover, Cd intake increased with advancing age due to the increase of DM intake as shown in Table (2). These results are in agreement with those obtained by El-Amary (1995), Salama (1995) and Gaafar *et al.* (2005) they found that Cd intake by calves increased with increasing the level of Cd in ration.

The intake of lead (Pb) by growing rabbits is shown in Table (4). Lead intake by rabbits increased significantly ($P<0.05$) with increasing the level of dietary Pb contamination. Which, rabbits in G5 revealed significantly (P<0.05) the highest Pb intake followed by those in G3, while those in G1, G2 and G4 had the lowest Pb intake and was nearly similar. Moreover, Pb intake increased with advancing age due to the increase of DM intake as shown in Table (2). These results are in accordance with those obtained by El-Amary (1995), Salama (1995) and Gaafar *et al*. (2005) they reported that Pb intake by calves increased with increasing the level of Pb in ration.

The concentration of Cd in plasma of growing rabbits is presented in Table (5). Cadmium concentration in plasma increased significantly (P<0.05) with increasing the level of dietary Cd contamination. Which, rabbits in G4 revealed significantly (P<0.05) the highest plasma Cd concentration followed by those in G2, while those in G1 had the lowest plasma Cd concentration. Also, plasma Cd concentration increased significantly (P<0.05)) with increasing the level of dietary Pb contamination. Moreover, plasma Cd concentration increased with advancing age due to the increase of dietar Cd intake as shown in Table (3). These results agreed with those obtained by El-Amary (1995), Phillips *et al*. (2003) and Gaafar *et al*. (2005) they found that there was an increase in cadmium concentration of blood with increasing feed cadmium concentration. Blood cadmium concentration increased gradually with increasing dietary lead.

The concentration of Pb in plasma of growing rabbits is presented in Table (6). Lead concentration in plasma increased significantly (P<0.05) with increasing the level of dietary Pb contamination. Which, rabbits in G5 revealed significantly (P<0.05) the highest plasma Pb concentration followed by those in G3, while those in G1 had the lowest plasma Pb concentration. Moreover, plasma Pb concentration increased with advancing age due to the increase of dietar Pb intake as shown in Table (4). These results are in accordance with those obtained by El-Amary (1995), Phillips *et al*. (2003) and Gaafar *et al*. (2005) they reported that there was an increase in blood lead concentration with increasing dietary lead.

The contents of Cd and Pb in liver, kidney and muscle of rabbits are shown in Table (7). The accumulation of Cd and Pb in liver, kidney and muscle of rabbits increased significantly (P<0.05) with increasing its levels in diets. These results are expectedly due to the high contents of these elemnts and subsequently the intake (Table 2). The higher accumulation of Cd and Pb were found in liver and kidney, however muscle showed the low accumulation of these elements. These results agreed with those obtained by Lopez Alonso *et al*. (2000 and 2003) and Gaafar *et al*. (2005) they reported that increasing Cd and Pb intake led to increasing the accumulation of Cd and Pb in liver, kidney and muscle. Phillips *et al*. (2003) found that there was an increase in cadmium concentration of all tissues and blood with increasing feed cadmium concentration, which was usually less when lead was also included in the feed. There was also an increase in tissue lead concentration with increasing dietary lead, and this was in most cases increased when cadmium was also included in the feed. The most sensitive tissues for cadmium and lead exposure were the kidney, liver, hair and teeth, and regression equations were developed for the accumulation rates in these tissues. Tissue and blood cadmium concentrations increased gradually with increasing dietary lead, whereas tissue lead concentration was not sensitive to dietary cadmium, except in the ribs and heart.

Table 1: Ingredients and chemical analysis of pelleted commercial diet.

Ingredient	%	Chemical composition	%
Yellow corn grain	18.00	DM	87.74
Barley grain	17.00	*Composition of DM %*	
Wheat bran	20.00	OM	92.34
Berseem hay	25.00	CP	17.56
Soybean meal (44%)	15.30	CF	12.41
molasses	3.00	EE	4.77
Limestone	1.00	NFE	57.60
Common salt	0.25	Ash	7.66
Premix*	0.30		
DL-methionine	0.15		
Total	100.00		

*one kg of premix contained 200,000 IU vit. A; 10,000 mg vit. E; 400 mg vit. B1; 1200 mg vit. B2; 400 mg vit. B6; 20 mg vit. B12; 400 mg vit. K3; 150,000 IU vit. D; 240 mg choline chloride; 400 mg pantothenic acid; 1000 mg niacin; 1000 mg folic acid; 40 mg biotin; 1700 mg manganese; 1400 mg zinc; 1500 mg iron; 600 mg copper; 20 mg selenium; 40 mg iodine; 8000 mg magnesium and calcium carbonate carrier to 1 kg.

Table 2: Average dry matter intake (gm/day) of growing rabbits during different experimental periods.

Age (week)	Experimental groups					SEM
	G1	G2	G3	G4	G5	
9-10	67.06	66.73	67.22	66.70	67.34	0.39
11-12	85.68	84.94	84.82	84.27	83.96	0.41
13-14	105.72	104.04	103.69	102.75	101.52	0.53
15-16	124.68	122.22	121.53	118.12	116.04	0.90
17-18	143.64^a	140.46^a	139.59^a	133.58^b	130.66^b	1.33
19-20	162.11^a	158.19^a	156.75^a	148.32^b	144.24^b	1.81
21-22	180.14^a	175.66^a	173.96^a	162.94^b	157.77^b	2.27
23-24	197.69^a	192.59^a	190.94^a	177.15^b	171.14^b	2.71
25-26	215.38^a	209.85^a	208.15^a	191.66^b	184.43^b	3.17
Overall mean	142.46^a	139.41^a	138.52^a	131.72^b	128.57^b	1.29

a, b: Means in the same row with different superscripts differ significantly (P<0.05).

Table 3: Average cadmium intake (mg/day) by growing rabbits.

Age (week)	Experimental groups					SEM
	G1	G2	G3	G4	G5	
9-10	0.019^c	0.033^b	0.019^c	0.058^a	0.019^c	0.004
11-12	0.024^c	0.042^b	0.024^c	0.073^a	0.024^c	0.005
13-14	0.030^c	0.052^b	0.029^c	0.089^a	0.028^c	0.006
15-16	0.035^c	0.061^b	0.034^c	0.103^a	0.032^c	0.007
17-18	0.040^c	0.070^b	0.039^c	0.116^a	0.037^c	0.008
19-20	0.045^c	0.079^b	0.044^c	0.129^a	0.040^c	0.009
21-22	0.050^c	0.088^b	0.049^c	0.142^a	0.044^c	0.010
23-24	0.055^c	0.096^b	0.053^c	0.154^a	0.048^c	0.011
25-26	0.060^c	0.105^b	0.058^c	0.167^a	0.052^c	0.012
Overall mean	0.040^c	0.070^b	0.039^c	0.115^a	0.036^c	0.008

a, b, c: Means in the same row with different superscripts differ significantly (P<0.05).

Table 4: Average lead intake (mg/day) by growing rabbits.

Age (week)	Experimental groups					SEM
	G1	G2	G3	G4	G5	
9-10	1.180^c	1.174^c	2.017^b	1.174^c	3.936^a	0.286
11-12	1.508^c	1.495^c	2.545^b	1.483^c	4.907^a	0.354
13-14	1.861^c	1.831^c	3.111^b	1.808^c	5.934^a	0.426
15-16	2.194^c	2.151^c	3.646^b	2.079^c	6.782^a	0.482
17-18	2.528^c	2.472^c	4.188^b	2.351^c	7.637^a	0.539
19-20	2.853^c	2.784^c	4.702^b	2.611^c	8.431^a	0.591
21-22	3.170^c	3.092^c	5.219^b	2.868^c	9.222^a	0.644
23-24	3.479^c	3.390^c	5.728^b	3.118^c	10.003^a	0.696
25-26	3.791^c	3.693^c	6.245^b	3.373^c	10.780^a	0.748
Overall mean	2.507^c	2.454^c	4.156^b	2.318^c	7.515^a	0.516

a, b, c: Means in the same row with different superscripts differ significantly (P<0.05).

Table 5: Cadmium concentration in blood plasma (ug/dl) of growing rabbits.

Age (week)	Experimental groups					SEM
	G1	G2	G3	G4	G5	
10	5.66^{d}	6.39^{b}	5.90^{cd}	7.18^{a}	6.07^{c}	0.14
12	5.67^{d}	6.72^{b}	5.91^{cd}	7.97^{a}	6.12^{c}	0.22
14	5.70^{d}	7.08^{b}	5.93^{cd}	8.86^{a}	6.13^{c}	0.31
16	5.71^{d}	7.45^{b}	5.95^{cd}	9.85^{a}	6.15^{c}	0.42
18	5.72^{d}	7.84^{b}	5.97^{cd}	10.94^{a}	6.18^{c}	0.53
20	5.75^{d}	8.26^{b}	6.03^{cd}	12.15^{a}	6.19^{c}	0.65
22	5.76^{d}	8.69^{b}	6.03^{cd}	13.51^{a}	6.20^{c}	0.80
24	5.77^{d}	9.15^{b}	6.04^{cd}	15.01^{a}	6.24^{c}	0.95
26	5.83^{d}	9.63^{b}	6.09^{cd}	16.67^{a}	6.26^{c}	1.13
Overall mean	5.73^{d}	7.91^{b}	5.98^{cd}	11.35^{a}	6.17^{c}	0.55

a, b, c: Means in the same row with different superscripts differ significantly (P<0.05).

Table 6: Lead concentration in blood plasma (ug/dl) of growing rabbits.

Age (week)	Experimental groups					SEM
	G1	G2	G3	G4	G5	
10	22.27^{c}	22.15^{c}	24.86^{b}	20.24^{c}	27.84^{a}	0.62
12	22.29^{c}	22.17^{c}	26.16^{b}	20.44^{c}	30.93^{a}	0.95
14	22.50^{c}	22.19^{c}	27.54^{b}	20.64^{c}	34.37^{a}	1.32
16	22.51^{c}	22.34^{c}	28.99^{b}	20.66^{c}	38.19^{a}	1.72
18	22.52^{c}	22.36^{c}	30.52^{b}	20.82^{c}	42.44^{a}	2.17
20	22.53^{c}	22.37^{c}	32.12^{b}	20.85^{c}	47.15^{a}	2.65
22	22.73^{c}	22.38^{c}	33.81^{b}	21.06^{c}	52.39^{a}	3.20
24	22.74^{c}	22.60^{c}	35.59^{b}	21.27^{c}	58.21^{a}	3.81
26	22.75^{c}	22.63^{c}	37.46^{b}	21.48^{c}	64.68^{a}	4.48
Overall mean	22.54^{c}	22.35^{c}	30.78^{b}	20.83^{c}	44.02^{a}	2.23

a, b, c: Means in the same row with different superscripts differ significantly (P<0.05).

Table 7: Cadmium and lead contents in liver, kidney and muscle (ppm on DM basis) of growing rabbits.

item	Experimental groups					SEM
	G1	G2	G3	G4	G5	
			Cadmium			
Liver	19.08^{c}	28.62^{b}	20.28^{c}	35.38^{a}	21.52^{c}	2.64
Kidney	36.80^{c}	51.70^{b}	37.70^{d}	74.30^{a}	39.16^{c}	3.81
Muscle	0.80^{c}	1.15^{b}	0.84^{c}	1.65^{a}	0.87^{c}	0.08
			Lead			
Liver	31.84^{c}	31.02^{c}	43.03^{b}	28.33^{c}	61.17^{a}	3.24
Kidney	35.03^{c}	34.13^{c}	47.33^{b}	31.17^{c}	67.28^{a}	3.56
Muscle	7.01^{c}	6.82^{c}	9.47^{b}	6.23^{c}	13.45^{a}	0.71

a, b, c: Means in the same row with different superscripts differ significantly (P<0.05).

Conclusion

From these results it could be concluded that cadmium and lead contamination led to increase the intake of cadmium and lead and their concentration in blood plasma and accumulation in body tissues of rabbits.

Correspondence to:
Gaafar H M A
Chief Researcher of Animal Nutrition, Animal Production Research Institute, Nadi El-Saied St., Dokki, Egypt.
E-mail: hamedgaafar@gmail.com

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