EFFECT OF HIGH MO-, CD-, PB-, HG- AND SE-CONTAINING CARROT ON RABBIT

HULLÁR I.[1], FEKETE S.[1], HUSZENICA GY.[1], KÁDÁR I.[2], SZILÁGYI M.[3], GLÁVITS R.[4], MÉZES, M.[5], KONCZ J.[2], BERSÉNYI A.[6]

[1] University of Veterinary Science
[2] Research Institute of Soil and Agrochemistry of the Hungarian Academy of Sciences
[3] Research institute for Animal Husbandry and Nutrition, Herceghalom
[4] National Veterinary Institute
[5] University of Agricultural Sciences, Gödöllő
[6] Research Institute for Small Animals, Gödöllő
H-1400 Budapest, P.O.Box. 2., Hungary

Abstract - Carrot was grown on soil polluted by heavy metal salts. The treatments in which a particular microelement reached high concentration (Mo 39.00%, Cd 2.30%, Pb 4.01%, Hg 30.00% and Se 36.20 mg/kg, respectively) in the carrot were selected from among the total of 13. Metabolic balance trial were carried out involving five male and female NZW rabbits. Following this blood samples were taken for the most important blood parameters to be determined. Animals were overslept by overdosing narcotics for the pathological examination which was followed by the measurement of the heart, liver, lungs, kidneys, spleen, ovaries/testicles and full digestive tract., adipose tissue, femur, hair, faeces and urine samples were taken for chemical investigation. In all the samples the Mo, Cd, Pb, Hg and Se content was determined. The digestibility of carrot with respect to all nutrients proved to be significantly better than that of the concentrate. Sample with high Pb content has the worst digestibility of the carrot samples, whilst with regard to crude fat the samples with high Cd, Hg, and Se concentrations had the lowest values. The impact of carrot samples containing Cd resulted in the weight loss of heart and liver. Microelements vary in their rate of accumulation in each of the organs: Mo and Cd in the kidneys; Pb in the kidneys, liver, bones and lungs; Hg in the kidneys and liver; Se in the liver, kidneys and heart accumulated the most. Mo and Se are lost from the body mainly through faeces and urine whilst Cd, Pb and Hg leaves the body almost exclusively through the faeces. A basic data is the proportion of elements lost from the body through the faeces and urine expressed as a percentage of the amount taken up: Mo 80.1 ppm; Cd 37.86 ppm; Pb 66.39 ppm; Hg 64.65 ppm and Se 47.41 ppm, respectively. Histological examinations revealed that the rate of spermiogenesis in the testis, compared to the controls, has been reduced in Mo, Cd, Pb and Hg Groups. Follicles containing healthy ova at different developmental stages were found in some of the Mo-Group, Se-Group and control does' ovaries, but signs of actual ovulations (corpus luteum) were not found. It should be noted that the impact of lead intake resulted in a considerable decrease in GGT with the increase of ALP activity. Both Cd and Hg had similar impacts on GGt and ALP activity. All experimental treatments decreased the activity of cholinestherase. ALT, AST, CK and kreatinine levels showed no real changes. Carrot diet containing microelements offered to animals improved glutation-peroxidase activity by 22-50% in the case of Mo, Cd and Se, while Pb decreased it by 18% and the impact of Hg resulted in no practical change.

INTRODUCTION

Besides lead, cadmium has been the object of numerous studies so far with special emphasis on heavy metal pollution. One of the many things that makes it a dangerous chemical is that it can get into the foodchain (SCHENKEL, 1988); its biological half-life is rather long (17 years for humans). Epidemiologic studies have showed correlation between cadmiumexpositio and the occurrence of human essential hypertonia and cadmium concentration in the adrenal gland. Cadmium taken up into the body binds to the methallotionein of the liver and kidneys. Cadmium could cause severe damages to the kidneys, testicles and ovaries (NRC, 1980). Because of different metallotionen concentrations as well as different ways in metabolism values obtained in different animal species are not convertible. Data for rabbits are especially poor. STOWE *et al.* (1972) carried out a 200 d trial in 3-month-old rabbits. It was found that treatments with 160 ppm Cd (in the form of $CdSO_4$) resulted in spleen atrophy, changes in blood parameters as well as damages to the liver and kidneys in rabbits. Oral administration of a single dose (43 mg/kg) of cadmium (in the form of $CdSO_4$) was found to be lethal by FAIRCHILD *et al.* (1977). Data on the impact of cadmium, taken up naturally by plants from the soil, on rabbits were not found in the research literature.

MATERIAL AND METHODS

Table 1 : Nutrient content of the basal diet and carrots containing microelements on air dry basis (%)

Nutrients	Basal diet	Carrot Mo	Carrot Cd	Carrot Pb	Carrot Hg	Carrot Se
DM	91,88	18,88	20,61	21,26	19,47	22,49
Air DM	91,88	90,12	91,63	90,76	89,09	91,52
Ash	7,03	4,93	5,46	5,07	5,11	5,39
OM	84,85	85,19	86,17	85,69	83,98	86,13
CP	15,20	10,60	10,30	9,80	11,10	11,10
CF	11,99	4,80	4,83	4,73	5,55	4,60
EE	1,80	2,70	1,78	2,28	2,28	2,08
NFE	55,86	67,09	69,26	68,88	65,05	68,35

Carrot samples (Table 1) were obtained from the ??terhelési tolerance experiments of MTA Research Institute of Soil and Agrochemistry, Nagyhörcsök. During the experimental period high doses (810 kg/ha) of Mo-, Cd-, Hg- and Se-fertilizers were applied to the soil and the rate of accumulation of the above heavy metals in carrots was determined. Those carrot samples of the 13 treatments were selected which showed considerable microelement concentrations : Mo 39.00 mg/kg, Cd 2.30 mg/kg, Pb 4.01 mg/kg, Hg 30.00 mg/kg and Se 36.20 mg/kg, respectively.

The experiment were carried out in the animal house, built for experimental purposes, of the Department of Nutrition, Veterinary University. The experiment involved 30 male and female NZW rabbits (2300 g average body weight), placed in individual hutches for metabolism experiment, to determine the apparent total tract digestibilities of the breeding rabbit concentrate used as a basal diet (Table 1). After determining apparent digestibilities the 30 rabbits were divided into six groups: one control and five experimental groups. The control group carried on feeding ad lib basal diet, while each of the five animals per experimental groups were forefed over a period of ten days. During this period daily basal diet intake was restricted to 50 g, meanwhile ad lib Mo-, Cd-, Pb-, Hg- and Se-containing carrots were offered. This was followed by a metabolism experiment using the "feeding mixture" of basal diet and carrot, and the calculation of digestibilities for nutrients in each of the carrot samples by the difference experimental formula (FEKETE and GIPPERT, 1982). Mixing ratio, as the real nutrients ratio in dry matter taken up from carrot, was calculated on the basis of basal diet analysis and of the fed and the remaining carrot samples. During both experimental periods daily total urine was collected from each of the animals. Following the metabolism experiment , after the 20 d feeding experiment of carrot samples, blood samples were taken from each of the animals to determine the following parameters at the Research Institute for Animal Breeding and Nutrition (Herceghalom) and at the University of Agricultural Sciences (Gödöllõ) : AST, ALT, GGT, ALP, CHE, CK, CHOL, TRIG, HS, CREA, malon-dialdehyde, glutathion-peroxidase, reduced glutation.

After measuring their body weights, animals were killed by overdosing narcotics for the pathological examination. Heart, liver, lungs, kidneys, spleen, ovaries/testicles and full digestive tract were measured. Appropriate samples of the above organs as well as of thyroid gland, femoral muscle, jejunum, ampulla ilei and breast bone were fixed in a buffered formaldehyde solution. Besides the above samples, adipose tissue, femur, hair, faeces and urine samples were taken for chemical investigation. Mo-, Cd-, Pb-, Hg- and Se-content were measured of each of the samples.

RESULTS

Table 2 shows the digestibility of nutrients in the basal diet and in treated carrots. Carrot diet met 43% of daily dry matter requirements, which meant the "feeding mixture" in the ?társult kihasználási experiment. These data provide evidence that the digestibility of carrots for each nutrients tended to be significantly better than those of the basal diet. Different carrot values showed that the worst digestibilities for crude protein were found in samples containing Pb, the lowest values for crude fat were found in samples containing Pb and Cd. No differences were observed in the rest of the parameters of nutrient digestibilities in carrot samples.

The impact of carrot samples containing Cd resulted in the weight loss of heart and liver. Table 3 summarizes the heavy metal content in the diet, urine, faeces as well as in the different organs and tissues. Parameters clearly show that microelements varies in their rate of accumulation for each of the organs : Mo and Cd in the kidneys; Pb in the kidneys, liver, bones and lungs; Hg in the kidneys and liver; Se in the liver, kidneys and heart.

Table 2 : Digestibility of the basal diet and carrots containing microelements, % (means ± s; n = 5)

Nutrients		Basal diet	Carrot Mo	Carrot Cd	Carrot Pb	Carrot Hg	Carrot Se
DM		74,80^{a}	81,64^{c}	79,24^{b}	80,11^{c}	78,78^{b}	79,81^{c}
	s	2,36	2,97	2,47	1,90	1,25	0,88
OM		75,07^{a}	82,25^{c}	80,03^{b}	80,61^{b}	79,56^{c}	81,37^{d}
	s	2,64	2,83	2,41	2,10	1,18	0,63
CP		75,63^{a}	79,81^{be}	78,34	75,57^{f}	78,45	77,47
	s	2,32	2,41	2,79	2,22	2,79	2,12
CF		28,63^{a}	42,56^{d}	38,02^{c}	44,91^{d}	39,12^{c}	43,44^{d}
	s	3,11	3,86	3,39	2,86	3,53	2,94
EE		72,17^{a}	81,27^{dg}	74,27^{he}	78,03^{cf}	74,75^{he}	75,22^{b}
	s	1,34	1,70	2,66	2,26	2,28	2,04
NFE		81,34^{a}	87,29^{d}	87,08^{d}	87,20^{d}	86,41^{d}	87,88^{d}
	s	1,21	1,96	1,54	1,43	0,90	0,81

DM= dry matter, OM= organic matter, CP= crude protein, CF= crude fiber, EE= ether extract, NFE= N-free extract, a-b:P<0.05 ; a-c : P<0.01 ; a-d:P<0.001 ; e-f : P<0.05 ; g-h :P<0.01

Table 4 shows what proportion of heavy metals taken up with the feed is removed from the body through urine and faeces and what proportion accumulates in the tissues. It can be seen that Mo and Se are lost from the body mainly through faeces and urine whilst Cd, Pb and Hg leaves the body almost exclusively through the faeces. Cd builds up in the tissues in largest quantities, which is followed by Se, Hg, Pb and Mo in decreasing order. A basic data is the proportion of elements lost from the body through the faeces and urine expressed as a percentage of the amount taken up which are the followings: Mo 80.18%; Cd 37.86%; Pb 66.39%; Hg 64.65% and Se 47.41%, respectively.

Table 3 : The Mo, Cd, Pb, Hg and Se content of the basal diet, carrots, feces, urine, and the different organs, ppm (n = 5)

	Basal diet Mo	Carrot Mo	Basal diet Cd	Carrot Cd	Basal diet Pb	Carrot Pb	Basal diet Hg	Carrot Hg	Basal diet Se	Carrot Se
Feed	0,527	39,000	0,140	2,300	1,580	4.010	0,0000	30,000	0,000	36,200
Faces	0,416	25,340	0,460	9,072	3,022	9,380	0,0000	32,060	0,000	11,703
Urine	0,417	6,602	0,000	0,004	0,040	0,046	0,0000	0,016	0,058	3,238
Hart	0,060	1,234	0,000	0,000	0,388	0,000	0,0000	0,000	0,584	19,400
Lungs	0,033	1,206	0,007	0,029	0,644	0,507	0,0000	0,000	0,727	14,748
Liver	1,262	1,878	0,119	0,722	1,717	1,846	0,0000	3,530	1,738	64,950
Kidney	0,754	3,464	1,119	2,590	0,042	4,664	0,0000	50,480	4,100	38,550
Spleen	0,000	1,083	0,012	0,000	0,759	0,151	0,0000	0,080	1,990	15,350
Adip.*	0,000	0,060	0,000	0,000	0,141	0,060	0,0000	0,000	0,000	0,634
Muscle	0,000	0,369	0,000	0,000	0,000	0,129	0,0000	0,132	1,327	13,490
Bone	0,000	1,202	0,000	0,000	0,000	0,649	0,0000	0,000	0,000	3,207
Hair	0,000	0,409	0,004	0,003	0,359	0,000	0,0000	0,000	1,371	2,695

*: adipose tissue

Table. 4 : Intake, excretion, and deposition of microelements and their proportion (n = 5)

		Intake	Excretion			Deposit
			by feces	by urine	All	
Mo, mg/day		1,398	0,362	0,759	1,121	0,241
	%	100,000	25,890	54,290	80,180	19,820
Cd, mg/day		0,103	0,039	-	0,039	0,064
	%	100,000	37,860	0,000	37,860	62,140
Pb, mg/day		0,241	0,157	0,003	0,160	0,081
	%	100,000	65,140	1,240	66,390	33,610
Hg, mg/day		0,116	0,055	0,020	0,075	0,041
	%	100,000	47,410	17,240	64,650	35,350
Se, mg/day		1,350	0,187	0,453	0,640	0,710
	%	100,000	13,850	35,550	47,41	52,59

The body weight measured directly before the slaughter was significantly smaller (P<0.05) in the experimental groups than in the controls (Table 5). It is likely to be accounted for by the fact that the dry matter uptake of the animals from the carrot is physically made difficult rather than by the heavy metal content of the carrot. It is also supported by the fact that the experimental group show no difference in this respect. Histological examinations revealed that the rate of spermiogenesis in the testis, compared to the controls, has been reduced in Mo, Cd, Pb and Hg Groups. Among spermatogenic cells, syncythial giant cells and degenerated cells indicating disorderly meiotic divisions were found to be abundant. Follicles containing healthy ova at different developmental stages were found in some of the Mo-Group, Se-Group and control does' ovaries, but signs of actual ovulations (corpus luteum) were not found.

Table 5 : The weight of the different organs, g (means ± s; n = 5)

Group		BW*	Hart	Lungs	Liver	Spleen	Kidney
Control		2790[a]	7,46[a]	11,56	77,19	1,58	15,56
	s	193	1,28	1,15	7,13	0,37	1,68
Mo		2484[b]	6,18	12,45	78,30	1,36	15,30
	s	124	0,85	2,24	6,56	0,10	0,93
Cd		2522[b]	6,02[b]	14,09	63,94[b]	1,42	15,19
	s	99	0,40	2,31	8,66	0,16	2,84
Pb		2496[b]	6,23	13,22	67,42	1,28	15,24
	s	134	0,75	1,30	9,63	0,17	1,65
Hg		2468[b]	6,08	13,67	80,24	1,36	14,56
	s	146	0,71	2,04	6,81	0,17	1,50
Se		2515[b]	6,16	13,56	78,90	1,31	14,46
	s	370	0,87	1,74	13,54	0,15	1,15

*: Body weight, a-b: P<0,05

With regard to changes in blood plasma parameters (Table 6.) it should be noted that the impact of lead intake resulted in a considerable decrease in GGT with the increase of ALP activity. Both Cd and Hg had similar impacts on GGt and ALP activity. All experimental treatments decreased the activity of cholinesthérase. ALT, AST, CK and kreatinine levels showed no real changes. Carrot diet containing microelements offered to animals improved glutation-peroxidase activity by 22-50% in the case of Mo, Cd and Se, while Pb decreased it by 18% and the impact of Hg resulted in no practical change (Table 7).

Table 6 : Blood parameters I. (means ± s; n = 5)

Group		AST U/l	ALT U/l	GGT U/l	ALP U/l	CHE U/l	CK U/l	CHOL U/l	TRIG g/l	GHS-Px U/g feh.	CREA µmol/l
Control		4,40	22,40	8,00	55,20	735	364,0	1,81	1,150	20,00	109,80
	s	1,01	4,49	9,36	31,10	365	90,3	0,31	0,300	8,17	9,32
Mo		5,20	12,80	3,54	96,20	476	623,0	1,51	1,06	13,20	111,60
	s	1,60	3,31	1,84	22,40	391	211,4	0,14	0,38	3,96	7,47
Cd		3,60	17,60	1,88	123,00	429	640,0	1,97	0,714	17,00	119,80
	s	1,49	12,05	1,34	27,90	224	90,6	0,18	0,237	5,83	6,55
Pb		4,20	11,60	3,88	102,60	385	646,0	2,21	0,938	15,00	106,80
	s	1,02	2,65	3,70	21,60	267	260,2	0,93	0,234	6,75	8,05
Hg		5,00	17,80	1,40	96,40	339	523,0	1,97	0,900	13,60	113,20
	s	1,26	7,24	0,38	6,97	585	82,7	0,36	0,208	10,20	15,79
Se		9,75	17,25	2,50	70,50	412	522,0	2,37	0,827	13,20	102,50
	s	5,97	2,86	1,75	12,80	258	224,0	1,39	0,117	0,82	4,15

AST= aspartate aminotransfererase, ALT= alanine aminotransferase, GGT= gamma glutyl-transferase, ALP= alcalic phosphatase, CHE= cholin-esterase, TRIG= triglyceride, GHSPx= gluthathion-peroxidase, CREA= creatinine

Table 7 : Blood parameters II. (means ± s; n = 5)

Group		Malon-di-aldehyde mmol/l	Glutation-peroxidase nkat/g ml	Glutation-peroxidase nkat/g protein	Reducted glutatione µmol/l	Reducted glutatione µmol/g
Control		2,12	2,20	2,48	109,87	1,41
	s	0,14	0,23	0,52	22,30	0,24
Mo		2,68	2,37	3,15	106,52	1,57
	s	0,30	0,44	0,47	10,52	0,29
Cd		2,65	2,30	3,71	116,74	2,25
	s	0,45	0,59	0,75	8,57	0,29
Hg		2,83	2,01	2,73	123,61	1,89
	s	0,64	0,16	0,40	6,20	0,26
Se		2,56	2,31	3,02	122,08	1,73
	s	0,43	0,10	0,38	2,70	0,36

DISCUSSION

On the basis of data obtained from this experiment it seemed that the negative effect of the above elements in high concentrations can hardly, if at all be monitored by determining the usual parameters (body weight gain, digestibility coefficients). Further examinations have revealed that it is the Cd that accumulates in the tissues to the greatest extent resulting in decreased heart and liver weight. From the fact that Cd, Pb and Hg almost exclusively lost through the faeces it can be concluded that the absorbed amount of the elements remain in the body and further excretion is very slow. The histological examinations showed that the accumulation of these elements in the animals' body is problematic in two respects: the spermiogenesis can be disturbed in breeding animals and in case of the slaughter animals the consumers could be endangered.

Generally the phosphorous uptake significantly reduced the activity of gamma-glutyl-transferase (GGT) and increased that of the alkali-phosphatase (ALP). Cadmium and mercury loading also increased ALP activity. These parameters indicate damaged kidneys (GGT) and bones (ALP) (GORECZKY and SÓS, 1983). All the treatments resulted in reduced activity of cholinesterase (CHE), which indicates the lesion of liver parenchyma (KANEKO, 1989). The level of astartate-aminotransferase (ALT, formerly GOT), alanine-aminotransferase (ALT, formerly GPT), kreatine-kinase in the blood and its kreatine content practically remained unchanged. Following the carrot diet containing Mo, Cd and Se the activity of glutation-peroxidase (GSH-Px) was increased by 20-50%, whilst the lead uptake decreased it. The carrot containing Hg had no practical effect on it. Changes in GSH-Px have showed that the body's immunity against free radicals has been altered by the experimental treatments.

REFERENCES

FEKETE S., GIPPERT T., 1982. Suggestion for standardization of rabbits' : metabolic experiments. (In Hungarian). Research report, Institute Anim. Breed. Nutr.

GORECZKY L., SÒS J., 1983. Clinical and chemical labiratory pocketbook. (In Hungarian), *Medicina*, Budapest.

FEHÉR J., VERECKEI A., 1985. Importance of free residue reactions in teh medical science. (In Hungarian) Biogal Pharmaceutical Factory, *Medicina*, Budapest.

KANEKO J.J. (ED.), 1989. Clinical Biochemistry of Domestic Animals. Forth Edition. *Academic Press. Inc. New York-London-Tokyo.*

NRC, 1980. Mineral Tolerance of Domestic Animals. National Academy of Sciences, Washington, D.C.

FAIRCHILD E.J., LEWIS R.J., TATKIN R.L.(Eds.), 1977. Registry of Toxic Effects of Chemical Substances. *DHEW Publ. No.* (NIOSH), 78-104-B., 2, 526.

STOWE H.D., WILSON M., GOYER R.A., 1972. Clinical and morphologic effects of oral cadmium toxicity in rabbits. *Arch. Pathol.*, 94, 389.

SCHENKEL,H.W., 1988. Nutritive Beeinflussung des Stoffwechsel verhalten von Cadmium bei Rind und Schwein. Habilitationsschrift. Hohenheim.