

SZENT ISTVÁN UNIVERSITY
Faculty of Veterinary Science

**Study of toxic metals (Cd, Pb, Hg and Ni) in rabbits and
broiler chickens**

PhD Thesis

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1. INTRODUCTION and AIM of STUDY

In international estimations, human health is affected by the environment in 25%. Considerable amounts of trace elements gets into the environment by the human activity. Industries, traffics and agricultural technologies are accounted for the largest discharge of trace elements into the water, soil and air. The disturbance of the biological cycle is mostly caused by the continuously increasing environmental pollution. Some of the pollutant elements are generally or partially essential for both plants and animals/humans, but each of them may have a mild to severe toxic effect.

Trace metals as the Cd, Pb and Hg are known to be toxic to all ecosystem components, both in the aquatic and terrestrial system. Since little or no recycling occurs, the amount entering the environment is continually accumulated. Consequently, the incorporation of these elements as well as Ni into the food chain of soil, plant, animal and man can lead to chronic diseases, reduce fertility, injure the cardiovascular and nervous system in both human beings and animals.

Classical examples for bio-accumulation and toxic effects in the food chain are the *itai-itai* disease (1970) for cadmium and the *Minamata* disease (1953) for mercury, both in Japan. Nowadays, after bursting of the reservoir dam belonged to a precious metal mine in Transylvania (Romania), the living world of the both rivers *Tisza* and *Szamos* in Hungary (2000) were badly damaged and thereby more hundred tons of fish died. Examination of their livers indicated that both Pb and Cd accumulated in the different fish species. Apart from the occupational exposure, thus the main source of heavy metal body burden for populations derives from food.

However, meanwhile significant steps have been taken to reduce toxic metal emission and dispersal to the environment, recently there has still been a growing concern at the accumulation of these elements in pasture soils.

Since in some areas, the accumulation of toxic metals in grazing ruminants, particularly in their offal, exceeds the permissible level. It is important to ensure that these organs and bone from such animals should not be included in products intended for animal (e.g. domestic pets) or human consumption.

Aim of study

1. Bio-monitoring the toxic elements (Cd, Pb, Hg and Ni) in the tissues and organs, especially the edible parts of the body of rabbits and broiler chickens.
2. Study their effects on growth and digestibility, depending on the ingestion way (dietary or oral administration).
3. Haematological and biochemical analysis as well as histopathological investigation of toxic metal burden of the bodies.
4. Differences between poultry and rabbit in response to Ni burden.
5. Interactions between Ni and other elements.

According to the purposes the following experiments were carried out:

- Experiment 1 (Feeding of carrots)
- Experiment 2 (Feeding of potatoes)
- Experiment 3 (Feeding of beetroots)
- Experiment 4 (Rabbit model for heavy metal loading)
- Experiment 5 (Broiler model for supplemental Ni)
- Experiment 6 (Rabbit model for supplemental Ni)

2. MATERIALS AND METHODS

2.1. Feeding processes, treatments

✓ Concentrations of toxic metals (in mg/kg DM)

	Cadmium (Cd)	
	Control	Contaminated
Soil	0.19	228.0
Carrots	0.15	2.3
Potatoes	0.2	2.12
Beetroots	0.4	4.72

	Lead (Pb)	
	Control	Contaminated
Soil	8.0	280.0
Carrots	1.9	4.01
Potatoes	0.6	4.1
Beetroots	<0.5	3.03

	Mercury (Hg)	
	Control	Contaminated
Soil	<0.5	61.0
Carrots	<0.5	30.0
Potatoes	<0.5	3.44
Beetroots	<0.5	6.75

	Nickel (Ni)	
	Control	Supplemented
Broiler diet	2.2	50 and 500
Rabbit diet	5.8	50 and 500

✓ *Experiment 1 (Feeding of carrots)*

“Basal diet” period: Rabbits (n=20) were given concentrate *ad libitum* for 14 days;

“Mixture ration” period: 50 g of concentrate and carrots containing Cd or Pb or Hg or uncontaminated were fed *ad libitum* for 14 days (n=5/treatment).

✓ *Experiment 2 (Feeding of potatoes)*

“Basal diet” period: Rabbits (n=16) were given 50 g of concentrate and 100 g of pelleted alfalfa meal for 14 days;

“Mixture ration” period: 25 g of concentrate and 50 g of alfalfa meal, and potato samples containing Cd or Pb or Hg or uncontaminated were fed *ad libitum* for 14 days (n=4/treatment).

✓ *Experiment 3 (Feeding of beetroots)*

“Basal diet” period: Rabbits (n=16) were given concentrate *ad libitum* for 14 days;

“Mixture ration” period: 50 g of concentrate and beetroots containing Cd or Pb or Hg or uncontaminated were fed *ad libitum* for 14 days (n=4/treatment).

✓ *Experiment 4 (Rabbit model for heavy metal loading)*

All rabbits (n=16) were fed *ad libitum* exclusively with concentrate. The animals (n=4/treatment) were orally treated with inorganic salts of Cd or Pb or Hg (3CdSO₄·8H₂O; Pb(NO₃)₂; HgCl₂) using concentrations based on Exp.1. The daily application of 0.2 ml of dissolved trace elements occurred through a metal catheter for 28 days. The control animals received daily 0.2 ml of distilled water.

✓ *Experiment 5 (Broiler model for supplemental Ni)*

The grower diet was supplemented with 0, 50 or 500 mg/kg Ni as NiCl₂·6H₂O between 14 and 49 days of age.

✓ *Experiment 6 (Rabbit model for supplemental Ni)*

The commercial pellet for rabbit supplemented with 0, 50 or 500 mg/kg Ni from NiCl₂·6H₂O were fed *ad libitum* for 24 days.

2.2. Investigated parameters and procedures

✓ Body weight (BW), feed intake (FI) and feed conversion efficiency (FCE): BW and FI of rabbits and broilers were weekly recorded; FCE of cockerels was calculated.

✓ Digestibility of nutrients: *Exp. 1-3* and *Exp. 6* (FEKETE and GIPPERT, 1983).

✓ Histopathology: Euthanasia of all rabbits was carried out by overdosing of intraperitoneal pentobarbital injection (*Exp. 1-3* and *Exp. 6*) and of 5 cockerels per treatment by inhalation of CO₂ (*Exp. 5*); heart, lung, liver, spleen, kidneys, gonads (ovaries or testicles) were completely recovered and appropriate samples of femoral muscle and rib bone were also taken. All samples were fixed in phosphate-buffered 10% formaldehyde solution, stained with haematoxylin and eosin and fat-red, and were examined by light microscopy (Central Veterinary Institute, Budapest). For enzyme analysis, the pancreas and the small intestine of rabbits were completely recovered and frosted (*Exp. 4*).

✓ Trace elements in the organs, tissues and fluids of the rabbits: Concentrations of Cd, Pb, Hg and Ni in the above-mentioned organs, adipose tissue, hair, blood, urine, faeces and soft faeces were detected by using a plasma emission spectrometer (ICP-AES) at the following wavelengths (nm): 228.802 for Cd, 220.353 for Pb, and 194.227 for Hg in *Exp. 1-3* (MTA TAKI) and by atomic absorption spectrometry using graphite furnace atomisation for Cd, Pb and Ni, and cold vapour technique for Hg in *Exp. 4*.

✓ Haematology and Zn-protoporphyrin (ZPP): For the determination of WBC (white blood cell), RBC (red blood cell), HGB (blood haemoglobin), HCT (haematocrit), MCV (mean cell volume), MCH (mean cell haemoglobin), MCHC (mean cell haemoglobin concentration), PLT (platelets) and for the ZPP-assay, at the 0 and 28 days of the *Exp. 4* blood samples (3 ml) were taken from the marginal ear veins of rabbits and were placed in plastic tubes containing K-EDTA as anti-coagulant.

The haematological values were measured (SZIU Fac. Vet. Sci., Dept. and Clinic of Internal Medicine, Budapest) by instrument of haem automat. The ZPP concentrations were measured by haematofluorometry of plasma (Institute of Public Health, Budapest). ✓ Serum biochemistry: Serum (2 ml) of each rabbit (*Exp. 1, 4* and *6*) and of 12 random broilers per treatment (*Exp. 5*) was used for determination of

✓✓ AST (EC 2.6.1.1.) and ALT (EC 2.6.1.2.) activity by optimised standard-method (BERGMEYER *et al.*, 1978);

✓✓ CK (EC 2.7.3.2.) activity by optimised standard-method (BRUNS *et al.*, 1976);

✓✓ ALP (EC 3.1.3.1.) activity by optimised standard-method (KAWADE, 1964);

✓✓ GGT (EC 2.3.2.2.) activity by kinetic colorimetric method (SZILÁGYI, 1990);

✓✓ CREA concentration by photometric colorimetric test with deproteinisation (Jaffé-reaction);

✓✓ GSHPx (EC 1.11.1.9.) activity by an end-point direct assay (MATKOVICS *et al.*, 1988);

✓✓ CHE (EC 3.1.1.8.) activity (SZILÁGYI, 1990);

✓✓ UREA concentration by UV kinetic determination (SZILÁGYI, 1990);

✓✓ CHOL and TRIG concentration by enzymatic colorimetric method (SZILÁGYI, 1990).

The biochemical parameters were measured (ÁTK, Herceghalom) by liquid reagent photometry using tests (Clinisotest).

- ✓ Pancreatic enzymes: For analysis both the pancreas and the contents of total small intestine were obtained (*Exp. 4*).
- ✓✓ Alpha-amylase (EC 3.2.1.1.) activity was measured by method of CESKA *et al.* (1969) using *Phadebas* Test;
- ✓✓ Trypsin (EC 3.4.21.4.) activity was measured by colorimetry (KAKADE *et al.*, 1969);
- ✓✓ Lipase (EC 3.1.1.3.) activity was measured by SCHÖN *et al.* (1961);
- ✓✓ Protein content was measured by the method of *Lowry-Folin* (HERD, 1971).

3. RESULTS

Body weight (BW), feed intake (FI), feed conversion efficiency (FCE). The final BW of rabbits consumed carrots, potatoes or beetroots was insignificantly smaller than that of animals (as controls) received only basal diet. The ingestion of toxic metals (Cd, Pb and Hg) did not reduce the FI of rabbits.

Supplemental 50 mg/kg Ni slightly (3%) improved the BW gain of broiler chickens compared to control animals. Supplemental 500 mg/kg Ni reduced the weight gain by 10% ($P < 0.05$) in broilers. The FI of cockerels was increased by supplemental 50 mg/kg Ni and decreased by 500 mg/kg Ni in comparison with controls. FCE was significantly worse by dietary 500 mg/kg Ni.

Supplemental 50 mg/kg Ni increased by 60% the daily weight gain in rabbits. Supplemental 500 mg/kg Ni reduced the weight gain by 20% in rabbits. Dietary supplementations of Ni (50 and 500 mg/kg) failed to reduce the FI of rabbits.

Digestibility of nutrients. The trace element content of root and tuber samples, generally, did not decrease the digestibility of major nutrients (e.g. crude protein) in comparison to uncontaminated samples.

Nickel - even of 500 mg/kg - failed to reduce significantly the digestibility of nutrients (e.g. crude protein) in rabbits. The decreased digestibility of crude fibre to approximately 20% is assumed to be within physiological ranges.

Trace element retention. About 38% of the ingested **Cd** was excreted from the rabbit body almost exclusively via the faeces and the total retention observed was about 62% of the ingested Cd. About 66% of the ingested **Pb** was excreted from the body; about 65% via the faeces and about 1% via the urine. Total retention observed in rabbits was about 34% of the ingested Pb. About 47.4% of the ingested **Hg** was excreted from the body almost exclusively via the faeces (47.2%) and only about 0.2% via the urine, and the total retention was about 52.6% of the ingested Hg. Considerable concentrations of toxic metals were also found in soft faeces.

Cadmium accumulated, in case of consuming Cd-exposed **carrots**, highly in the kidneys (double of normal kidneys), followed by the liver (6-fold of the control). Cadmium could not be detected in the heart, lung, spleen, testicles, adipose tissue, muscle, ribs and hair. In case of consuming Cd-contaminated **potatoes**, Cd was retained mainly also in the kidneys (3-fold of the control) and liver (4-fold of the control). Detectable amount of Cd was found in the testicles (twice of control) and ribs. Cadmium could not be detected in the heart, lung, spleen, adipose tissue, muscle and hair. In case of consuming Cd-contaminated **beetroots**, Cd accumulated mainly also in the kidneys (double of the control) and less in the liver. Cadmium could not be detected in heart, lung, spleen, testicles, adipose tissue, muscle, ribs and hair.

Lead accumulated, in case of consuming Pb-exposed **carrots**, in the kidneys (10-fold of the control). Detectable amount of Pb was found in the liver and ribs. Lead could not be detected in the heart, lung, spleen, testicles, adipose tissue, muscle and hair. In case of consuming Pb-contaminated **potatoes**, the markedly elevated concentrations of Pb were found in the kidneys and the spleen (twice the control). There was not detectable amount of Pb in the heart, lung, liver, testicles, adipose tissue, muscle, ribs and hair. In case of consuming Pb-contaminated **beetroots**, the Pb could not be detected in any organs and tissues.

Mercury accumulated, in case of consuming Hg-exposed *carrots*, in the kidneys (100-fold of the control) and the liver (7-fold of the control). Mercury could not be detected in the heart, lung, spleen, testicles, adipose tissue, muscle, ribs and hair. In case of consuming Hg-contaminated *potatoes*, Hg was retained only in the kidneys. In case of consuming Hg-contaminated *beetroots*, Hg could be also detected only in the kidneys.

After *orally administered Cd* the highest levels were measured in the kidneys (5-fold of the control) and liver (7-fold of the control), while the lowest one in the muscle. The considerable Cd content in the lung, spleen, ovaries and bone was 2-3-fold, while in the hair it was 10-fold that of the controls. The detectable amount of Cd in muscle (heart and skeletal) was as much as in controls.

Lead concentrations observed in heart, liver, lung, muscle and ribs were 2-5 times higher than the Pb concentrations in these tissues of control animals.

Mercury content was increased in all tissues examined, even in the fat depot. Comparing to the control animals, markedly elevated concentration of Hg was observed in the kidneys, liver, ovaries and hair. Mercury could be detected even in muscle and ribs.

In case of **Ni**-burden, approximately 98% of Ni was eliminated from the body via faeces and 0.5-1.5% with the urine, and approximately 1% was retained in the body.

With increasing the Ni-load, the Ni content of the organs was significantly increased including the muscle. Nickel accumulated in the kidneys, ribs, heart, liver and in the lung. The ovaries had relatively high concentration of Ni (0.3 or 0.4 mg/kg DM, 50 and 500 mg/kg Ni, respectively), being 3-4 times higher than that of control animals.

Histopathology. Histological examination in each experiment revealed that the rate of spermatogenesis in the testis was reduced in the **Cd**- and **Pb**-loading groups compared to rabbits fed uncontaminated samples. In case of consuming **Hg**-contaminated carrots, the rate of spermatogenesis in the testis was also reduced. A large number of syncytial giant cells and degenerated cells indicating abnormal meiosis were found among the spermatogenic cells.

In case of does, ovarian follicles containing healthy ova at different developmental stages were found, but signs of actual ovulation (*corpus luteum*) were not found.

Orally administered toxic metals (i.e. Cd, Pb, Hg) caused lesion of liver parenchyma such as focal fatty infiltration and slight tubulonephrosis in rabbits. Histological examination of bones estimated that any of the toxic metal burdens of rabbit body failed to damage the function of bone marrow and did not alter the *erythropoiesis*.

Mild or moderate form of pathological focal fatty infiltration caused by 50 or 500 mg/kg **Ni** was found in the liver of *broilers*.

Ovarian activity in *rabbits* was reduced by adding 500 mg/kg Ni. Nickel burden of body failed to affect the Cu, Zn, Fe and Mn concentration of the different organs in rabbit.

Haematology. Due to the oral administration, the **Pb** concentration in serum doubled, while the ZPP concentration remained unchanged. RBC, HGB and HCT are significantly decreased (by 13, 20 and 11%, respectively), while MCH and MCV are increased (by 9 and 4%) by Pb burden. These haematological data indicate that macrocytic hyperchromic anaemia has developed in Pb-treated rabbits.

Serum biochemistry. The biochemical results confirmed the changes in liver and/or kidneys found in toxic metal-burden rabbits. The increased activity of both AST and ALT and the reduced activity of CHE (50-60% of the control) are indicating the damage of the liver parenchyma. Both the reduced activity of GGT and the increased activity of ALP indicate toxicity of trace elements to the kidneys and/or liver. The increased concentration of CREA could be a result of the tubulonephrosis developed in Cd-burden rabbits.

Nickel supplementations, generally, did not have any significant effect on enzyme activities in broiler chickens and rabbits.

Pancreatic enzymes. Orally administered trace elements, especially **Pb** and **Hg**, reduced the activity of pancreatic enzymes.

4. NEW SCIENTIFIC RESULTS

1. The accumulation of toxic metals (i.e. Cd, Pb and Hg), and their effect on the digestibility of nutrients, biochemical and histopathological changes (in the liver, kidneys, testes) are treatment-dependent, namely, it depends not only on the character of the pollutant, but also on the matrix of the feedstuff.
2. Orally administered Cd increases the serum ALT activity; Pb increases the AST activity and Hg increases both the ALT and AST activity in rabbits. The investigated toxic metals (i.e. Cd, Pb, Hg) reduce the activity of pancreatic enzymes.
3. Toxic metals (i.e. Cd, Pb, Hg) reduce the rate of spermatogenesis in testes, resulting in reproductive impairment of male rabbits. Nevertheless, they fail to inhibit the ovarian activity of female rabbits.
4. Under the experimental condition of sub-acute Pb burden, the ZPP concentration remain practically unchanged in rabbits.
5. In case of Ni burden, approximately 98% of Ni is eliminated from the body of the rabbits via the faeces and 0.5-1.5% with the urine and approximately 1% is retained in the body. With increasing the Ni-load, the Ni content of muscle and ovaries increases. Dietary 500 mg/kg Ni inhibits the ovarian activity in female rabbits.
6. Supplemental 50 mg/kg of Ni improves the weight gain by 3% in broilers, while the 500 mg/kg of Ni reduces the weight gain by 10% and the feed conversion efficiency in broiler cockerels.
7. Even 50 mg/kg of Ni damages the liver parenchyma induces pathological focal fatty infiltration in broilers and rabbits.
8. Nickel burden of body failed to affect the Cu, Zn, Fe and Mn concentration of the different organs in rabbits.

5. LITERATURES

5.1. List of original publications based on this dissertation

1. **Bersényi, A.**, Fekete, S., Hullár, I., Kádár, I., Szilágyi, M., Glávits, R., Kulcsár, Margit, Mézes, M., Zöldág, L. (1999): Study of the soil-plant (carrot)-animal cycle of nutritive and hazardous minerals in a rabbit model. *Acta Vet. Hung.*, 47:(2):181-190.
2. Fekete, S. Gy., **Bersényi, A.**, Kádár, I., Glávits, R., Koncz, J., Zöldág, L. (2001): Study of the soil-plant (potato and beetroot)-animal cycle of nutritive and hazardous minerals in a rabbit model. *Acta Vet. Hung.*, 49:(3):301-310.
3. **Bersényi, A.**, Fekete, S., Szócs, Z., Berta, Erzsébet (2003): Effect of ingested heavy metals (Cd, Pb and Hg) on haematology and serum biochemistry in rabbits. *Acta Vet. Hung.*, 51:(3):297-304.

5.2. Other scientific publications

1. **Bersényi, A.**, Hullár, I., Fekete, S., Huszenicza, Gy., Kádár, I., Koncz, J., Szilágyi, M., Glávits, R., Mézes, M. (1997): Cd-, Pb-, Hg-és Se-szennyezett talajon termesztett burgonya etetésnek vizsgálata házinyúlon (in Hungarian). In: Szendrő Zs. (Szerk.): 9. Nyúltenyésztési Tudományos Nap előadásai, PATE, Kaposvár, pp. 107-114.
2. **Bersényi, A.**, Hullár, I., Fekete, S., Huszenicza, Gy., Kádár, I., Szilágyi, M., Glávits, R., Mézes, M., Koncz, J. (1997): Feeding effect of potatoes grown up on soil polluted with Cd, Pb, Hg and Se on rabbit. *Proc. 17. Mengen- und Spurenelemente, Jena*, pp. 112-117.
3. **Bersényi, A.**, Hullár, I., Fekete, S., Huszenicza, Gy., Kádár, I., Szilágyi, M., Glávits, R., Mézes, M., Koncz, J. (1998): Nagy Se-, Mo- és nehézfém (Cd, Pb és Hg)-szennyezetségű talajon termesztett sárgarépa hatásának vizsgálat nyúltakarmányozási kísérletben (in Hungarian). In: Cser Mária Ágnes, Sziklainé László Ibolya (Szerk.): A szelén szerepe a környezetben és egészségvédelemben. MTA, Bp., pp. 94-99.
4. **Bersényi, A.**, Fekete, S., Glávits, R., Gaál, T., Hudák Aranka (2001): A házinyúl nehézfém (Cd-, Pb- és Hg-)-terhelésének klinikai laboratóriumi diagnosztikája (in Hungarian). In: Szendrő Zs. (Szerk.): 13. Nyúltenyésztési Tudományos Nap előadásai, PATE, Kaposvár, pp. 107-112.

