PhD. THESIS

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STUDIES ON THE LEAD CONTAMINATION OF FORAGES IN HUNGARY AND ITS EFFECT ON RUMINANTS

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1. PRELIMINARIES AND TARGETS

For thousands of years man has been a subordinate, partly defenceless member of the ecosystem, because his existence is determinately dependant on natural environment.

With the development of natural sciences man has deliberately overcome his natural environment and has been changing it with different interferences. Heavy metals play - in this respect too - an important role in the production technologies of the developed countries. Some of them (e.g. zinc, copper) are vital for life processes of animals and plants, maintenance or increase of agricultural productivity, and fulfilling essential microelement requirements of man.

At the same time a number of heavy metals has gained a world-wide reputation as a dangerous source of pollution to environment. Studies on heavy metal circulation of soil and food chain came into lime-light in the past years, as the consequence of both; the expand of environmental protective approach in science as well as in public; and the development and higher punctuality of analytical methods and instrumental examinations.

I focused my work on 3 topics:

1. Determination of lead content in crops. I analyzed the lead content of major roughages and forages grown in Hungary, and forages grown in industrial areas, and along busy roads respectively.

2. Analyses on lead exposure of dairy cattle. I studied the lead content of milk and hair samples in agricultural areas and in the vicinity of busy roads.

3. The effect of artificial lead exposure on sheep to identify the effect of lead load level on
   - the digestibility of forages
   - the lead level in indicator organs, and
   - the activity of some enzyme complexes of ruminants
   - analyses on the lead penetration ratio through placenta subject to lead exposure, and the level of its appearance in newborn lambs.

2. EXPERIMENTAL MATERIAL AND METHOD

Examinations on the lead content of plants

I collected feed samples - green grass, green alfalfa, cornsilage, silaged grass, silaged alfalfa, hay, alfalfa hay, beet -slice - from 9 agricultural areas.

My examinations lasted 3 years. Sample-taking spots were far from industrial areas and busy roads. Furthermore I analyzed the lead content of major forages such as corn, wheat, barley, sunflower, soybean, pea, full-fat soya, rapeseed, fishmeal, mixed animal protein meal.

From the vicinity of busy motorways (M1, M7, M5, M3), main roads, and industrial areas (Dunaújváros, Százhalombatta, Ózd, Miskolc, Pécs, Komló) 63 wheat, 18 barley, 53 alfalfa, 61 grass, 20 sugar-beet samples were collected and their lead level was examined. Green wheat and barley and green alfalfa samples were taken from the immediate vicinity of the road (3 m), and 50 and 100 metres off the road.

Sugar-beet root and leaf samples were taken from the immediate vicinity (3 m), and from a distance of 10, 20, 30 and 50 metres off motorway M1.

In the case of green alfalfa I measured to what extent the concentration of lead and microelements (iron, copper, zinc and manganese) changes by tapwater washing. Washing was carried out by a rainlike shower of 20 minutes.

Examination on the lead content of milk and hair

Examinations were carried out in 9 farms, where the lead content of major forages was measured. Some small farmers were also involved, whose animals were grazed near busy roads, or fed hay from such places or near industry.

Examination of artificial lead addition with female and male lambs.

Two utilization and lead exposure trials were carried out in succession: in trial 1 and trial 2, 9-9 Hungarian merino sheep females and males were included. The lambs were kept individually in metabolism cages. Their feed ration was elaborated in line with their daily vital needs of 810 alfalfa hay and 330 maize grits. Females and males were accustomed to feed for 12 days prior to the trial start.

Three groups were formed with 3 animals in each:

Lead exposure trial 1:

Group I.	Control (just basic feed)
Group II.	Basic feed +50 mg lead/day
Group III.	Basic feed+500 mg lead/day

Lead exposure trial 2:

Group I.	Control (just basic feed)
Group II.	Basic feed+10 mg lead/day
Group III.	Basic feed+500 mg lead/day

In both trials lead was mixed to maize grits in lead-acetate form. During examination utilization trials were carried out in succession, continuously in 4 phases.

In phase 1 prefeeding lasted 12 days, collection phase lasted 5 days. This time the 3 groups received feed without lead addition. Partly the results of this phase formed the basis of comparison too. In the next 3 phases group II and group III received lead addition in the above quantity. In both trials, at every group, nutrient absorption was measured after the feeding on days 28, 47 and 62. Collection phase of both trials lasted 5 days.

I determined the apparent digestibility of examined nutrients. On the 17^{th}, 28^{th}, 47^{th} and 62^{nd} day after the trials start wool sample and blood sample from venajugularis were taken, the serum was separated, and stored on -20 C^{o} till utilization.

In trial 1 mortality of the trial groups exposed to lead was 1 animal of each (on day 40 and 53 of lead exposure). Cause of mortality in both cases was urinal calculus and a consequent uremia. At the end of the trial the animals were euthanized. In both trials I took organ samples and rumen content of the slaughtered as well as the died animals, and

determined their lead-, manganese-, copper- and zinc content, while in the case of rumen content only the lead content was determined.

The following parameters were examined in connection with the trials:

- the effect of lead load on digestibility
- the effect of lead load on the lead content level of certain indicator organs (liver, kidney, brain, wool)
- the effect of lead load on the activity of some enzymes (kreatinkinase, glutamil-transferase, aspartat-aminotransferase, kolin-esterase, delta-aminoleukulinacid, dehydrogenase)
- the effect of lead load on the colesterol and the creatinin content of blood.

Examination on the effect of artificial lead exposure in ewes and newborn lambs.

The animals originated from sheep farms, where the level of lead contamination was within tolerance. I synchronized the animals' heat, then they were artificially inseminated. They were involved into trial after the successful fertility that was determined by ultrasonic pregnancy check.

4 groups were formed with 4 ewes in each. Group 1 is the control, that received normal feed without lead addition. Group 2 received 10 mg lead addition (equal to the highest acceptable natural level), while group 3 received 50 mg, group 4 250 mg of lead addition in lead-acetate form

mixed in the daily feed ration. During my experiments ewes were fed 2,2 kg maize silage, 0,3 kg hay and 1,0 kg ewe feed.
In each group the lambs of ewes were euthanized in the very moment of birth and analysed. My purpose was to measure the extent of lead penetration through placenta.

Examinations on the chemical composition of feed applied during animal trials.

Dry material	Msz 6830/3-77
Crude protein	Msz 6830/4-74
Raw fat	Msz 6830/6-78
Raw fibre	Msz 6830/7-78
Raw ash	Msz 6830/8-78

Lead determination was carried out by atomicabsorption method on the basis of Msz 6830/33-82 standard. Preparation of samples for examination was uniform; atomicabsorbtion was carried out on 283,0 nm wave length, atomization in graphite owen.
Determination of other microelements (Cu, Zc, Mg) was also carried out by atomic absorption method of MSz. Plant and organ samples were dried on 60 C^o then on 105 C^o till mass stabilization, then incinerated on 450 C^o in quartz pot.
Ash was dissolved in 10 pct hydrocloric acid, after condensation, again dissolved in hydrocloric acid, filtered into a test tube and filled up till

mark. From basic solution prepared this way, I identitified copper on 324,7 nm, zinc on 213, 9 nm and manganese on 297,5 nm wave length.
After drying lead content of organ samples was measured, having distroyed in microwave destroyer.

Preparation of rumen content.

Homogenized rumen content was dried first on 60 C^o to mass stabilization, then 5 grms were weighed into a quarz pot and incinerated on 450-500 C^o, and lead was measured after nitric acid treatement by ETA techniques.

Determination of zinc-, copper-, manganese- and lead content of wool sample.

The wool sample was first degreased by organic solvent, then incinerated on 450 C^o, finally exposed by hydrochrolic acid and nitric acid respectively.

Determination of zinc-, copper- and lead content of milk samples.

50 ml of milk sample was evaporated, then incinerated, after exposition by hydrochloric acid, iron and zinc were directly determined.
In milk and organ samples lead was determined after exposition in a microwave destroyer.

Following chemical analyses were applied for the determination of biochemical parameters:

- CK, C 2.7.3.2. NAC-activated, UV optimalized kinetic standard method
- GGT, EC 2.3.2.2. colorimetric method, substrate, 1 - g - glutamil - p - nitroanilid
- AST, EC 2.6.1.1. uv, optimalized standard method,
- CHE, EC 3.1.1.8. colorimetric method, substrate, butiril-tiokolin.
- Kreatinin: colorimetric, on the basis of jaffe-reaction
- Cholesterol: CHOD-PAP; enzymatic colorimetric method
- ALA-D, EC 4.7.1.24. method

Analyses were carried out according to the recommendations of Deutsche Gesellschaft für klinische Chemie and International Society for Animal Clinical Biochemistry, Boehringer and Clinisotest make agents, and Eppendorf ACP 5040 type instruments were used.

Applied statistical analysis

The effect of different feed and lead load trials was analysed by significancy examination, applying t-test.

Nutrient value of feedstuffs, where digestibility was determined, was calculated with the coefficients from the utilization trials.

3. CONCLUSIONS

- Lead content of plants from the examined agricultural areas was showed to be under the critical level (5 mg/kg). Lead contamination of plants from industrial areas and near to busy roads was higher than that of plants from agricultural areas. Occasionally these levels proved to be higher than the critical point. Lead contentration of plants seemed to show significant decreases with the distance from the road. At a distance of 100 metres lead content was found similar to that of plants collected from agricultural areas. By washing the plants with water the level of lead contamination significantly decreased, suggesting that some proportion of the contamination originates from the surface of the plants.

- Lead and microelement content of milk samples from cows grazing along busy roads was higher than that of the milk from agricultural areas. There was a high correlation between the lead content of milk and hair.

- Lead contamination of 50 or 500 mg/animal/day to the feed had not any influence on the digestibility of nutrients. Neither level nor length of lead exposition had significant influence on digestibility of nutriens.

- Addition of 500 mg Pb to the diet significantly increased lead concentrations in liver, kidney, brain and wool. Cu level in liver and brain was decreased by the dietary exposure of 500 mg/animal/day of

lead exposure, that may be the consequence of the antagonistic effect of copper to the lead.

- Lead exposure resulted in changes in the activites of enzymes tested in the experiments. These findings indicate that lead contamination initiates tissue damages in the animals.

- Dietary lead contamination of pregnant ewes caused excretion of Pb in the amniotic fluid and significant concentrations of lead could be detected in the indicator organs of newborn lambs. This may demonstrate that lead can be penetrated through the placenta.

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