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Effect of lead in water on the absorption of copper, iron, manganese and zinc by sheep (*Ovis aries*) infected with sheep tapeworm (*Moniezia expansa*)

I. Jankovská [a,*], J. Száková [b], D. Lukešová [c], I. Langrová [a], P. Válek [a], J. Vadlejch [a], Z. Čadková [a], M. Petrtýl [a]

[a] Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, 165 21 Praha 6, Suchdol, Czech Republic
[b] Department of Agroenvironmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, 165 21 Praha 6, Suchdol, Czech Republic
[c] Department of Animal Science and Food Processing in Tropics and Subtropics, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, 165 21 Praha 6, Suchdol, Czech Republic

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ABSTRACT

The sheep tapeworm (*Moniezia expansa*) and its host *Ovis aries* were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) for their copper, iron, manganese, zinc and lead levels. Element concentrations in cestode parasites were compared to those in various organs (liver, kidney, and muscle) of sheep. Tapeworms in the small intestine of sheep that were administered 2 g of $Pb(CH_3COO)_2$ per os daily (7 days) had significantly higher lead concentrations than sheep tissues. Cu levels significantly increased after Pb administration in sheep muscle and sheep tapeworms. Contrarily, Zn content significantly decreased in sheep muscle, but significantly increased in sheep tapeworms. However, Mn content significantly decreased after Pb administration in sheep tapeworms. Furthermore, Fe content significantly decreased after Pb administration in sheep liver and kidneys.

1. Introduction

Toxic elements in the environment can also increase the susceptibility of affected individuals to infections. Several helminths are capable of accumulating considerable element concentrations from the host body (Baruš et al., 2003; Lafferty, 1997; Sures, 2004). It remains unclear whether element accumulation of the parasitic worm affects element levels in the tissues of the definitive host; very few comparative studies on element concentrations in tissues of infected and uninfected hosts are available. Only Sures et al. (1998) has worked with cattle (*Bos primigenius* f. *taurus*) parasitized by the digenean *Fasciola hepatica*, while Sures et al. (2000) has researched the pig species (*Sus scrofa* f. *domestica*) parasitized by the archiacanthocephalan *Macracanthorhynchus hirudinaceus*.

A very common farm animal (*Ovis aries*) and its common tapeworm (*Moniezia expansa*) were selected for the present study. Sheep were also chosen due to the fact that there are very few models for studying element concentrations in parasite infected hosts under farming conditions. Sheep live and feed for the greatest part of the year outdoors, and therefore, the concentration of heavy metals in this species reflects the environmental load more accurately than in other productive animals (Antoniou et al., 1995). The targeted edible body tissues that accumulate of the most potentially toxic elements (PTEs) are the liver and kidneys. The mechanism of metal absorption blood, and the presence or absence of antagonistic metals can interact to influence the rate and extent of heavy metal accumulation in edible body tissues (Wilkinson et al., 2003).

This experiment investigates the absorption of zinc, manganese, iron, copper, and lead by sheep infected with an intestinal parasite (*M. expansa*), and affected by inorganic lead added to water. Concentrations of those elements were determined in the sheep tapeworm *M. expansa* as well as in the kidney, liver, and muscle of infected or uninfected sheep exposed to experimental amounts of lead.

2. Materials and methods

2.1. Sheep

The sheep (*O. aries*) used were the Oxford Down breed from a small ecological farm in western Bohemia. The sheep were approximately 6 months old, male, and weighed 20–25 kg.

2.2. Animal welfare

The experiments were performed in compliance with current laws of the Czech Republic (§17 of the Act No. 246/1992 coll. on Protection Animals against Cruelty in present statues at large). The Central Commission for Animal Welfare (CCAW) – Faculty of Agrobiology, Food and Natural Resources (FAFNR), Czech

* Corresponding author.
E-mail address: jankovska@af.czu.cz (I. Jankovská).

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University of Life Sciences Prague (CULS) approved all experimental protocols. We have also attached a certificate of competency according to §17 of the Act No. 46/1992 coll. on the Protection of Animals against Cruelty, presented to Jaroslav Vadlejch, Ph.D. (co-author of manuscript).

2.3. Experimental design

All the sheep were randomly divided into four groups ($n = 6$); the first group (Pb) contained uninfected, Pb exposed sheep; the second group (TPb) contained tapeworm infected and Pb exposed sheep; control group (T) contained tapeworm-infected, unexposed sheep.

Experimental sheep were naturally infected with *M. expansa* groups without *Moniezia* infection were dewormed by albendazole (Aldifal®, Mevak). Coprological examinations were carried out using the modified floatation – concentration method by Breza (1957), which entails the following: solution A (1000 g $MgSO_4{\cdot}7H_2O$ crystalline + 1000 ml hot H_2O); solution B (2000 g $Na_2S_2O_3{\cdot}7H_2O$ + 1000 ml hot H_2O). Combining three parts of solution A + three parts of solution B + one part water (for 1.25–1.30 g/cm^3 solution density).

The intensity of infection in sheep was 20–25 *Moniezia* eggs per 1 g of feces. This level was similar for all animals. At necropsy, there were approximately five tapeworms found in the small intestine. Additional parasites discovered during the coprological examination included *Trichostrongylus* spp. (3–6 eggs in 1 g of feces) and *Trichuris* spp. (1–5 eggs in 1 g of feces).

Lead solutions were prepared by dissolving 2 g of $Pb(CH_3COO)_2$ in 10 ml of distilled water, and administered orally to the sheep every day for a period of 1 week (7 days).

2.4. Sampling and analytical procedure

After exposure, the sheep were slaughtered and dissected. Samples of muscle, liver, and kidney as well as parasites were taken with stainless-steel scissors and forceps, which had been cleaned beforehand with double-distilled water. For the stabilization of blood samples, ethylene diamine tetraacetic acid (EDTA) was applied. The cestodes were removed from the intestine using the instruments mentioned above. All samples were frozen at −26 °C until further processing and freeze-drying (Lyovac GT-2, Germany).

Pressurized wet ashing: an aliquot (~500 mg of dry matter) of the liver, kidney, muscle, and tapeworm samples were weighed in a digestion vessel. Concentrated nitric acid (8.0 ml) (Analytika Ltd., Czech Republic), and 30% H_2O_2 (2.0 ml) (Analytika Ltd., Czech Republic) were added. The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave assisted wet digestion system for 30 min at 220 °C. After cooling, the digest was quantitatively transferred into a 20 ml glass tube and filled to volume with deionized water. A certified reference material BCR 185R Bovine Liver was applied for the quality assurance of analytical data. In this material, the certified value of elements were as follows: 0.172 ± 0.009 mg kg^{-1} Pb, 277 ± 5 mg kg^{-1} Cu, 11.07 ± 0.29 mg kg^{-1} Mn, and 138.6 ± 2.1 mg kg^{-1} Zn. In our experiment, however, the values for this material were as follows: 0.178 mg kg^{-1} Pb, 286 mg kg^{-1} Cu, 11.8 mg kg^{-1} Mn, and 135 mg kg^{-1} Zn. The total content of Pb, Cu, Fe, Mn, Zn in the digests was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia). For the determination of low lead content flameless atomic absorption spectrometry (GFAAS, VARIAN AA280Z, Varian, Australia equipped by GTA-110Z graphite furnace atomizer) was applied.

2.5. Statistical analysis

Element concentrations (Cu, Fe, Mn, Zn) were compared among tissues and treatments using the Kruskal–Wallis test, while the Mann–Whitney U-test was utilized for tapeworms. All computations were carried out using the Statistica ver. 9 program (Statsoft, USA).

3. Results and discussion

We examined the associations of lead exposure with micronutrient (Cu, Fe, Mn, Zn) concentrations in sheep infected with tapeworm (Table 1, Figs. 1–4). The toxicity of lead is attributed to the fact that it interferes with the normal function of a number of enzymes. Bipolar Pb forms strong bonds with enzymes bearing sulfhydryl groups, thus inhibiting their action (Bryce-Smith and Stephens, 1983). We examined the influence of lead exposure and cestode infections on micronutrient concentrations in sheep and sheep tapeworm.

The need for an adequate supply of microelements derives from their vital role in animal health. Microelements have a number of structural, catalytic and regulatory functions in the organism, and they also play a major role in the immune system. Toxic elements pose major health risks due to their high bioaccumulation potential, persistent nature and harmful biological effects (Sharma et al., 2010).

The experimental doses given "2 g of $Pb(CH_3COO)_2$ daily (7 days)" were not what would be normally experienced under field conditions, they were much greater. This is because 2–3 g of $Pb(CH_3COO)_2$ is toxic for humans and 20–25 g is lethal (live weight of experimental sheep was 20–30 kg).

Information regarding the impact of parasites on metal uptake by their hosts is contradictory. In our study, sheep with cestode

Table 1
Mean concentrations of Cu, Fe, Mn, Zn, and Pb in the sheep tissues (kidney, liver, muscle) and sheep tapeworm *Moniezia expansa* (mg/kg of dry weight) after 1 week Pb exposition.

Tissue	Group	Cu	Fe	Mn	Zn	Pb
Kidney	T	16.20	406.08^{k}	4.08	95.96	0.63^{kk}
	TPb	14.32	144.45^{k}	4.26	77.62	19.60^{kk}
	Pb	14.26	328.22	3.93	80.46	39.17^{kk}
Liver	T	261.30^{l}	524.54^{ll}	8.37^{lll}	113.10	0.52^{llll}
	TPb	244.77	174.73^{ll}	7.26	101.87	16.58^{llll}
	Pb	145.23^{l}	319.99	5.44^{lll}	127.47	22.05^{llll}
Muscle	T	2.86^{m}	39.52	0.11^{mm}	173.67^{mmm}	0.17
	TPb	3.96^{m}	45.36	0.32	109.47^{mmm}	0.18^{mmmm}
	Pb	4.51^{m}	58.13	0.70^{mm}	82.60^{mmm}	0.60^{mmmm}
Tapeworm	T	3.77^{t}	12.35	9.85^{tt}	66.38^{ttt}	0.14^{tttt}
	TPb	8.75^{t}	14.72	6.54^{tt}	83.38^{ttt}	78.95^{tttt}

Group (Pb) contained uninfected, Pb exposed sheep; group (TPb) contained tapeworm infected and Pb exposed sheep; group (T) contained tapeworm-infected, unexposed sheep. ^{k,l,m,t}Indicate statistical differences ($P \leqslant 0.05$).