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10.2478/s11687-011-0033-3

HELMINTHOLOGIA, 48, 4: 237 – 243, 2011

Competition for minerals (Zn, Mn, Fe, Cu) and Cd between sheep tapeworm (*Moniezia expansa*) and its definitive host sheep (*Ovis aries*)

I. JANKOVSKÁ[1*], D. LUKEŠOVÁ[2], J. SZÁKOVÁ[3], I. LANGROVÁ[1], J. VADLEJCH[1], Z. ČADKOVÁ[1], P. VÁLEK[1], M. PETRTÝL[1], M. KUDRNÁČOVÁ[1]

[1*]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, E-mail: *jankovska@af.czu.cz*; [2]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; [3]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

Summary

Concentrations of various essential and toxic elements (Zn, Mn, Fe, Cu and Cd) were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) in the sheep tapeworm (*Moniezia expansa*) and in different tissues of its host *Ovis aries.* The element concentrations of the cestode parasites were compared to different organs (liver, kidney, and muscle) of sheep that were exposed to experimental amounts of Cd (0.2 g of $CdCl_2$ added to 10 ml of distilled water and administered orally to the sheep every day for a period of 1 week). All sheep were randomly divided into four groups; the first group (Cd) contained uninfected, Cd exposed sheep, and its control (group C) were uninfected and unexposed to Cd; the second group (TCd) contained infected, Cd exposed sheep, and its control (group CT) contained infected, unexposed sheep. The experimental Cd exposure resulted in significantly higher Mn concentrations in sheep tapeworms (10.0 mg/kg) than in sheep muscle (0.6 mg/kg) and kidney (0.8 mg/kg). The experimental Cd exposure also significantly decreased the Cu concentrations in sheep liver and muscle. Moreover Cd exposure decreased the Fe concentrations in sheep kidney but caused it to increase in sheep liver and muscle. Zinc concentrations showed no differences between groups (Cd, TCd, C, T) in any monitored sheep tissues. The article also discuss the effect of tapeworm infection on a significant decrease of Fe in sheep muscle, liver and kidneys, as well as a decrease in Cu levels of the muscles and liver. This mineral imbalance may contribute to various health problems such as osteoporosis, metabolic processes disorder, antioxidant (SOD) dysfunction etc.

Keywords: Cd exposure; Zn; Mn; Fe; Cu; sheep; tapeworm

Introduction

Cadmium (Cd) belongs to a group of toxic heavy metals, which can cause adverse effects in the organisms even at very low concentrations. Cd is a ubiquitous environmental polutant and carcinogenic metal. Animal and human epidemiologic studies have pointed to cadmium as a potential novel risk factor for cardiovascular disease, the leading cause of death worldwide (Menke *et al.*, 2009). The findings of Lee *et al.* (2011) suggest that cadmium in blood may be associated with an increased risk of ischemic heart disease and hypertension.

We examined the associations of cadmium exposure and cestode infections with micronutrients 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. Contrarily, the use of drugs in food-animal production results in residual contamination of food products, as well as environmental contamination associated with disposal of wastes from the animals (Silbergeld & Nachman, 2008). Toxic elements are major health risk concerns due to their high bioaccumulation potential, persistent nature and harmful biological effects (Sharma *et al.*, 2010).

Cadmium has high mobility in soil-plant systems and gets accumulated easily into the food chain. A bioindicator reflecting Cd availability in agricultural environments could be relevant for human dietary Cd exposure. Sheep are usually fed locally produced foyer crops, and crops are the main source of Cd intake in humans. Sewage sludge is a major source of Cd on grassland (Wilkinson *et al.*, 2003) and it may cause the accumulation of potentially toxic metals by grazing ruminants. Consumption of Cd contaminated feed or water may result in Cd-induced damage to the intestinal mucosae, causing disturbances to the element absorption in that region of the gastrointestinal tract (Phillips *et al.*, 2005).

Cd exposure can cause an imbalance of essential elements such as Cu, Fe, Mn and Zn. Noel *et al.* (2004) described subchronic dietary exposure of rats to cadmium alters the metabolism of metals essential to bone health. Also, Šimonyté *et al.* (2010) reported many effects of Cd action reset from interactions with necessary micro- and macro-elements, especially Ca, Zn, Cu, Fe and Se.

Heavy metals in environment also can increase the susceptibility of affected individuals to infections.

Several helminths are able to accumulate considerable concentrations of elements from the host body (Baruš *et al.*, 2003; Lafferty, 1997; Sures, 2004). It remains unclear if the element accumulation of the parasitic worms affects the 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. A very common farm animal (*Ovis aries*) and their common tapeworm (*Moniezia expansa*) were selected for the present study. Sheep were also chosen considering the fact that there are very few models for studying element concentrations in a parasite infected host under farming conditions. Only Sures *et al.* (1998) worked with cattle (*Bos primigenius* f. *taurus*) parasitized by the digenean *Fasciola hepatica,* and Sures *et al.* (2000) with the pig species (*Sus scrofa* f. *domestica)* parasitized by the archiacanthocephalan *Macracanthorhynchus hirudinaceus* determined the influence of these parasites on element concentrations in the host tissues.

This experiment investigates the absorption of essential elements by sheep with an intestinal parasite (*Moniezia expansa*) affected by inorganic Cd added to water. The concentrations of Zn, Mn, Fe, Cu, and Cd were determined in the sheep tapeworm *Moniezia expansa* and in the kidney, liver and muscle of infected or uninfected sheep exposed to experimental amounts of cadmium.

Material and methods

Sheep

The sheep (*Ovis 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.

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).

Central Commission for Animal Welfare (CCAW) - Faculty of Agrobiology, Food and Natural Resources (FAFNR), Czech University of Life Sciences Prague (CULS) approved all experimintal protocols.

We attached also the certificate of competency according to §17 of the Act No. 246/1992 coll. on Protection Animals against Cruelty in present statues at large for Jaroslav Vadlejch, PhD (co-author of manuscript).

Experimental design

All sheep were randomly divided into four groups (n = 6); the first group (Cd) contained uninfected, Cd exposed sheep, and its control (group C) were uninfected and unexposed to Cd; the second group (TCd) contained infected, Cd exposed sheep, and its control (group CT) contained infected, unexposed sheep.

Experimental sheep were naturally infected with *Moniezia expansa* (groups without *Moniezia* infection were dewormed by albendazolum (Aldifal®, Mevak). The coprological examinations were carried out using the Breza flotation method.

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

Cadmium solutions were prepared by dissolving 0.2 g cadmium chloride ($CdCl_2$) in 10 ml of distilled water, and administered orally to the sheep every day for a period of one week (7 days).

Sampling and analytical procedure

After exposure, the sheep were slaughtered and dissected. Samples of muscle, liver, kidney and blood as well as parasites were taken with stainless-steel scissors and forceps, which had been cleaned beforehand with double – distilled water. For stabilization of blood samples, ethylene diamine tetraacetic acid (EDTA) was applied. The cestodes were removed from the intestine using the same instruments. 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 sample 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 topped up (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 represented 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 286 mg.kg^{-1} of Cd, 11.8 mg.kg^{-1} of Mn, and 135 mg.kg^{-1} of Zn was determined in this material. The total content of

Table 1. Comparison of tapeworm and sheep tissues accumulation (*, **, *** P < 0,05)

group	element	mg/kg dry weight	mg/kg dry weight	mg/kg dry weight	mg/kg dry weight
T	**Cd**	muscle (0.004)*	**tapeworm (0.01)**	liver (0.4)	kidney (0.6)*
TCd	**Cd**	muscle (0.7) *, **	kidney (14.6) **	**tapeworm (18.3)**	liver (23. 8)*
T	**Cu**	muscle (3.7)*	**tapeworm (5.2)**	kidney (16.8)	liver (48.1)*
TCd	**Cu**	muscle (3.3)*	**tapeworm (5.0)****	kidney (15.4)	liver (43.0)*,**
T	**Mn**	muscle (1.2)*	kidney (3.5)	**tapeworm (6.6)**	liver (9.0)*
TCd	**Mn**	muscle (0.6)*, **	kidney (0.8)***	**tapeworm (10.0)****	liver (15.1)*,***
T	**Fe**	**tapeworm (8.9)**	muscle (35.3)*	kidney (156.2)	liver (237.1)*
TCd	**Fe**	**tapeworm (8.0)**, ***	muscle (125.7)*	kidney (245.9) **	liver (515.6)*, ***
T	**Zn**	**tapeworm (85.2)**	kidney (106.4)	muscle (118.9)	liver (167.8)
TCd	**Zn**	**tapeworm (80.9)***	muscle (110.3)	kidney (111.1)	liver (161.4)*

Cd group: uninfected, Cd exposed sheep, and its control (group C) with uninfected and unexposed to Cd;
TCd group: infected, Cd exposed sheep, and its control (group CT) with infected, unexposed sheep

Cd, Cu, Fe, Mn, Zn in the digests was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia).

Statistical analysis

The concentrations of elements (Zn, Cu, Fe, Mn) were compared among tissues and treatments using using the Kruskal-Wallis test. All computations were done using program Statistica ver. 9 (Statsoft, USA).

Results

We examined the associations of cadmium exposure with micronutrients (Zn, Fe, Cu, Mn) concentrations in sheep and sheep tapeworm (*Moniezia expansa*). Table 1 illustrates the differences in concentrations of Cd, Cu, Fe, Mn and Zn in sheep tissues and sheep tapeworms influenced by experimental Cd exposure (group TCd).

Sheep tapeworms accumulated more Mn and Cd than did sheep kidneys and muscle. The Cu concentration was also higher in sheep tapeworms than in sheep muscle (Tab. 1). Only Fe and Zn concentrations were higher in sheep tissues (muscle, kidney, liver) than in sheep tapeworms. This is in contrast to lead accumulation wherein sheep tapeworms accumulated a much higher quantity of lead than did sheep tissues (Jankovská *et al.*, 2010a) and similarly in fish intestinal helminth (*Acanthocephalus lucii*) was lead accumulation always higher than in the host (*Perca fluviatilis*) tissues (Jankovská *et al.*, 2011; Turčeková *et al.*, 2002).

Informations regarding the impact of parasites on the metal uptake by their hosts are contradictory. In the experimental study of Jankovská *et al.* (2010 b) on the cadmium uptake by *Moniezia expansa,* no reduction of the cadmium concentration in the host tissues was observed. Sheep with *Moniezia expansa* infection always exhibited higher cadmium concentrations in the tissues (with the exception of blood) than their uninfected conspecifics. It appears that *Moniezia expansa* do not reduce cadmium accumulation in host tissues as was determined with lead accumulation (Jankovská *et al.* 2010a). The highest mean cadmium concentrations were found in the liver of sheep infected with *M. expansa* (24.5 ± 11.5 $mg.kg^{-1}$ dry weight). The mean cadmium concentration measured in *Moniezia expansa* was 21.5 ± 19.2 $mg.kg^{-1}$ dry weight, which was 31 and 1.5 times higher than levels determined in the muscle and kidney of host respectively. However, the above mentioned concentrations was 0.9 times lower than levels determined in the liver of the host.

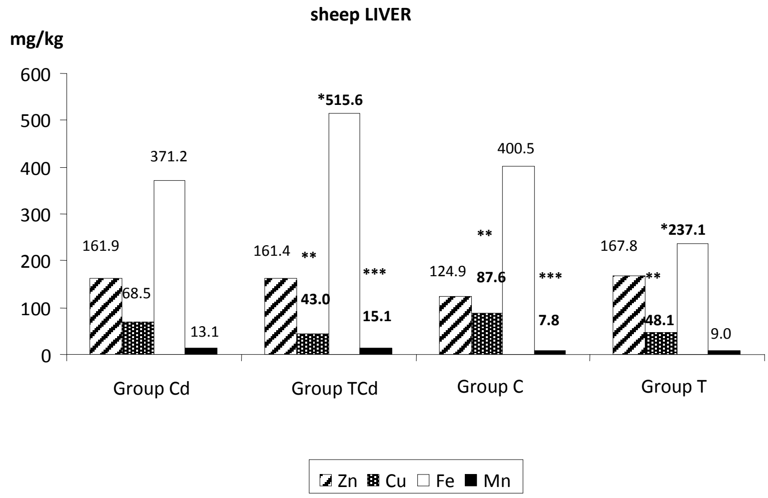

Fig. 1: The concentrations of Zn, Cu, Fe, Mn in sheep liver
(group Cd: uninfected sheep with Cd exposure; group TCd: tapeworms infected sheep with Cd exposure; group C: uninfected sheep without Cd exposure; group T: tapeworms infected sheep without Cd exposure)
Different number of asterisks indicates significang difference between compared groups:
*Fe, **Cu, ***Mn significant difference P < 0.05

The concentrations of Zn, Cu, Fe, Mn in sheep liver:
Fe: TCd > C >Cd > T (515.6 – 237.1);
Zn: T > Cd ≥ TCd > C (167.8 – 124.9);
Mn: TCd > Cd > T > C (15.1 – 7.8);
Cu: C > Cd > T > TCd (87.6 – 42.9)
The presence of sheep tapeworm with Cd exposure (group TCd) increased the levels of Fe and Mn, however, it reduced Cu levels in sheep liver; these sheep liver Cu levels were negatively correlated to burden of sheep (Fig.1). Moreover, Mn concentrations were two times higher in the most stressed sheep (group TCd) than in the livers of the control (unexposed) sheep.

The concentrations of Zn, Cu, Fe, Mn in sheep kidney:
Zn: C > Cd > TCd > T (116.2 → 106.39);
Cu: C > T > Cd > TCd (17.87 → 15.39)
Fe: C > TCd > Cd > T (287.69 → 156.25);
Mn: TCd ≥ C > Cd > T (6.85 → 3.55)
In the kidney of sheep (Fig. 2), the highest Zn, Fe, Cu, Mn concentrations were observed in control group C (sheep without tapeworms and Cd exposure). Conversely, the lowest Zn, Fe, Mn concentrations were found in the kidneys of sheep with tapeworms infections (group T). As Sures (2002) described, this can be due to competition for minerals between a parasite and its definitive host.

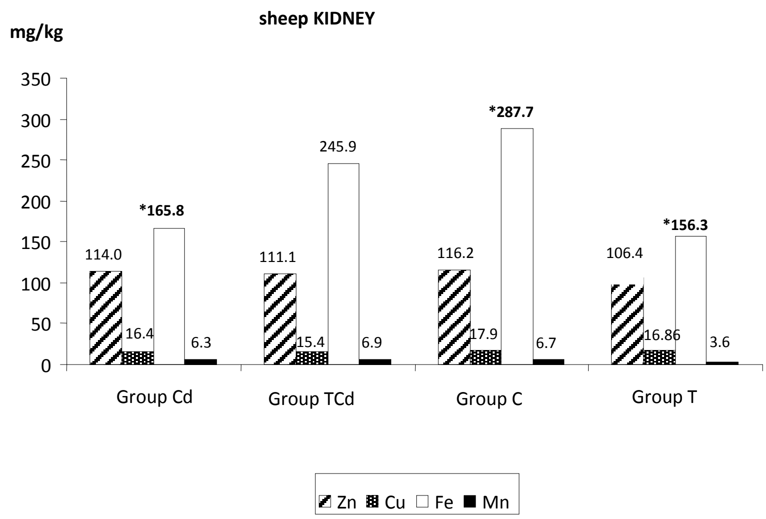

Fig. 2: The concentrations of Zn, Cu, Fe, Mn in sheep kidney
(group Cd: uninfected sheep with Cd exposure; group TCd: tapeworms infected sheep with Cd exposure; group C: uninfected sheep without Cd exposure; group T: tapeworms infected sheep without Cd exposure)
*Fe significant difference P < 0.05

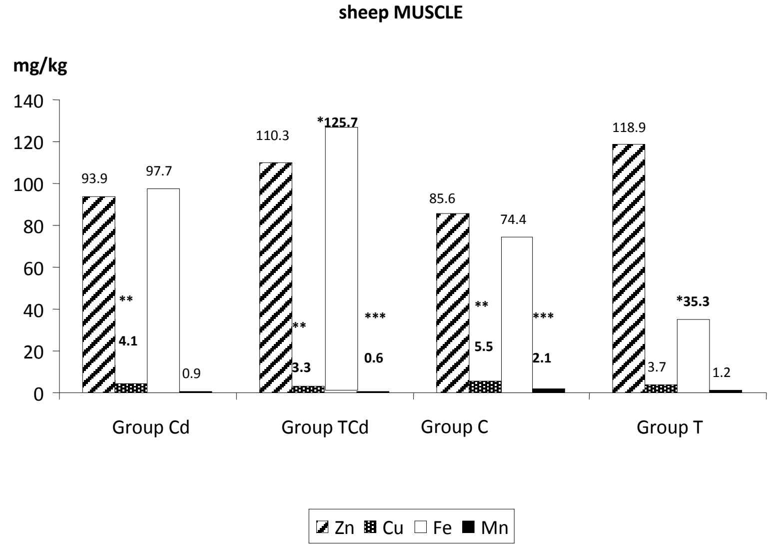

Fig. 3: The concentrations of Zn, Cu, Fe, Mn in sheep muscles
(group Cd: uninfected sheep with Cd exposure; group TCd: tapeworms infected sheep with Cd exposure;
group C: uninfected sheep without Cd exposure; group T: tapeworms infected sheep without Cd exposure)
Different number of asterisks indicates significant difference between compared groups:
*Fe, **Cu, *** Mn ($P \leq 0.05$)

The concentrations of Zn, Cu, Fe, Mn in sheep muscle:
Mn: C > T > Cd > TCd (2.08 → 0.56);
Zn: T > TCd > Cd > C (118.95 → 85.58)
Cu: C > Cd > T > TCd (5.48 → 3.28);
Fe: TCd > Cd > C > T (125.72 → 35.29)
The highest Cu and Mn concentrations were in the muscle of sheep without tapeworms or Cd exposure (control group C, Fig. 3). The lowest Cu and Mn concentrations were in the muscle of sheep most burdened with tapeworm and Cd exposure (group CdT, Fig. 3). Levels of Fe were affected the most; they were shown to be significantly higher in cadmium exposed sheep than in sheep without cadmium exposure. Fe concentrations were highest in the muscle of the most burdened sheep (CdT) followed by sheep with Cd exposure (group Cd).

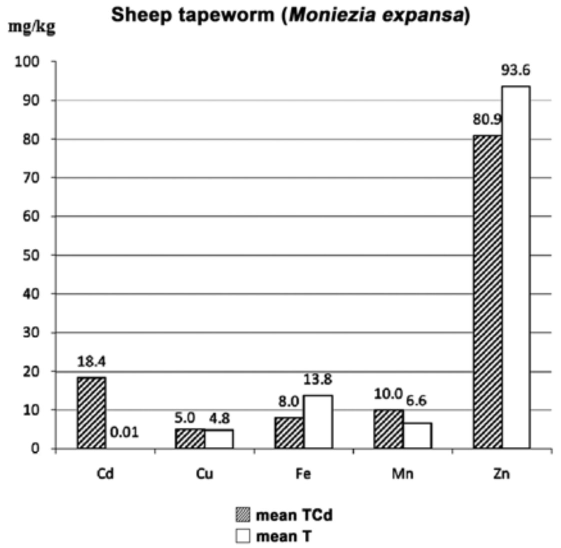

Fig. 4. The concentrations (mg/kg dry weight) of Zn, Cu, Fe, Mn in sheep tapeworm (*Moniezia expansa*)
(group TCd: tapeworms infected sheep with Cd exposure;
group T: tapeworms infected sheep without Cd exposure)
Zn and **Fe:** T > TCd; **Mn** and **Cu:** TCd > T

Discussion

Sures (2002) observed many elements (Ba, Ca, Co, Cu, Fe, Mg, Mn, Sr, Zn and Al, Ag, Cd, Cr, Ni, Pb, Tl) at significantly higher concentrations in the acanthocephala than in the host tissues. In our study, all elements (Cd, Cu, Fe, Mn and Zn) concentrations were higher in sheep tissues (especially in the liver) than in sheeptapeworms. Only in group T were Cd concentrations the highest in the kidneys. The lowest element concentrations were in the muscle (Cd, Cu, Mn) and tapeworms (Fe, Zn).
The experimental exposure of cadmium (groups Cd, TCd) and sheep tapeworms (groups T, TCd) significantly influenced levels of copper, ferrum and manganese in muscle, kidney and liver of sheep (Table 1; Figs. 1, 2, 3, 4). Significant differences among levels of all elements (Zn, Cu, Fe, Mn) in examined tissues individual treatments were found ($P < 0.05$).
In sheep muscle, Mn and Cu concentrations were the highest in control group C (sheep without tapeworms and Cd exposure, Fig. 3). Tapeworm infections and Cd exposure indicated Mn and Cu consumption at stress stimulants. Thielen *et al.* (2004) described that concentrations of the essentials elements (Mg and Mn) in the muscle of the host (*Barbus barbus*) were positively correlated with the total number of parasites in the gut. In our study, sheep infected with tapeworm showed an increase in Zn concentration in the muscle (group T and TCd, Fig. 3). With respect to Cd exposure, there was an increased Fe concentration in the muscle (TCd and Cd groups).

Alonso *et al.* (2004) observed that Cd levels were positively correlated with Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se and Zn levels in the kidney of cattle, and with Ca, Co and Zn levels in the liver of cattle. However, Swiergosz-Kowalewska and Holewa (2007) and Noel *et al.* (2004) documented that cadmium decreased the iron concentrations in the liver whereas zinc concentrations increased in the liver of *Clethrionomys glareolus* and *Rattus norvegicus*, respectively. Contrarily, in our experiments only Zn levels in sheep tissues were unaffected by Cd exposure or tapeworm infections. Tapeworm infection resulted in significant Fe decreasing in sheep kidney compared with control group C.

Conclusions

The experimental Cd exposure resulted in significantly higher Mn concentrations in sheep tapeworms (10.0 mg/kg) than in sheep muscle (0.6 mg/kg) and kidney (0.8 mg/kg).
The experimental Cd exposure also significantly decreased the Cu concentrations in sheep liver and muscle. Moreover Cd exposure decreased the Fe concentrations in sheep kidney but caused it to increase in sheep liver and muscle.
Zinc concentrations showed no differences between groups (Cd, TCd, C, T) in any monitored sheep tissues. Tapeworm infection resulted in a significant decrease of Fe in sheep muscle, liver and kidneys, as well as a decrease in Cu levels of the muscles and liver.
This mineral imbalance may contribute to various health problems such as osteoporosis, metabolic processes disorder, antioxidant (SOD) dysfunction etc.
Thus, more investigations are necessary to determine how intestinal parasites are able to affect element levels in host tissues.

Acknowledgements

This work was supported by project no. 111A199 of the National Agency for Agricultural Research. We would also like to thank Mr. Brian Kavalir for his proofreading services.

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RECEIVED JUNE 8, 2011 ACCEPTED SEPTEMBER 5, 2011