DOES ZINC OVERDOSE IN RAT DIET ALTER CU, FE, MN, AND ZN CONCENTRATIONS IN A TAPEWORM HOST?*

I. Jankovská[1], V. Sloup[1], J. Száková[2], J. Magdálek[1], B. Horáková[1], I. Langrová[1]

[1]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Zoology and Fisheries, Prague, Czech Republic
[2]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Agroenvironmental Chemistry and Plant Nutrition, Prague, Czech Republic

We evaluated Cu, Fe, Mn, and Zn concentrations in the bone, muscle, testes, intestine, liver, kidneys and tapeworm parasites *Hymenolepis diminuta* of rats from four groups: 12 animals given zinc lactate (120 mg/rat and week) in feed mixture (M0 group); six animals given zinc lactate (120 mg/rat and week) in feed mixture and infected with tapeworms (MT group); six control animals fed a standard mixture of ST-1 for rats (00 group); and six control animals fed a standard mixture of ST-1 for rats and infected with tapeworms (0T group). The experiment was conducted over a six-week period. In our study, tapeworm presence decreased element concentrations in the majority of rat tissues. Tapeworms accumulated higher levels of zinc and manganese than did the majority of host tissues; however, they accumulated very little iron and copper in comparison to the host tissues. Zinc overdosing increased manganese concentrations in rat tissues; zinc overdosing also seemed to protect the liver from absorption of Fe by tapeworms.

Hymenolepis diminuta, *Rattus norvegicus*, accumulation, zinc lactate, manganese, iron, copper

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INTRODUCTION

Risk element contamination of the environment is a global problem (Gil-Jimenez et al., 2017; Kim et al., 2017; Vymazal, 2017). Using a large number of manufactured products with a wide range of applications is becoming more common. This has resulted in the general population becoming increasingly exposed to a wide variety of xenobiotics that may cause adverse health effects (Jimenez-Diaz et al., 2016; Kulma et al., 2017). Studies dealing with animal nutrition are still required due to increasing environmental contamination (Burges et al., 2016; Henriquez-Hernandez et al., 2016; Pavlovic et al., 2016), which affects food quality. Among the widely discussed elements, zinc belongs to the most intensively investigated ones, because of the worldwide utilization of this element resulting in potential contamination of the environment (Strachel et al., 2016).

Zinc is an essential trace element necessary for normal human functioning. It serves as an enzyme cofactor and protects cell membranes from lysis caused by complement activation and toxin release (Sloup et

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al., 2017). Zinc is not stored in the body; therefore, dietary intake is required. Zinc lactate is a zinc salt of lactic acid. This product aids in the digestion and metabolism of phosphorus, and is necessary for protein synthesis and blood stability. Zinc lactate is commonly used as a dietary supplement for both humans and animals (S l o u p et al., 2016).

Parasitic diseases are very common in animals, especially in farm animals bred in high concentrations; parasitoses can be very dangerous (K y r i a n o v a et al., 2017). However, some parasites as intestinal helminths can be also beneficial to their hosts. It is known that cestodes and acanthocephalans can decrease heavy metal concentrations in a host body (S u r e s et al., 2002; S u r e s , 2004).

We worked with experimental animal models (laboratory rats: *Rattus norvegicus* var. *alba*) infected with rat tapeworms (*Hymenolepis diminuta*); rats were later given high doses of zinc lactate, which is commonly used by humans as a dietary supplement. The aim of this research was (*i*) to determine how tapeworms affect the accumulation of zinc, and (*ii*) to assess the potential shifts of other micronutrients (Cu, Fe, Mn) in a host given a zinc overdose.

Hypothesis: tapeworms decrease element concentrations in a host given Zn overdoses.

MATERIAL AND METHODS

This experiment was conducted over a six-week period with thirty male Wistar rats divided into the following groups: twelve animals given zinc lactate (120 mg/rat and week) in feed mixture (M0 group); six animals given zinc lactate (120 mg/rat and week) in feed mixture and infected with tapeworms (MT group); six control animals fed a standard mixture of ST-1 for rats (00 group); and six control animals fed a standard mixture of ST-1 for rats and infected with tapeworms (0T group). The experimental design and animals used in this study are described in detail in J a n k o v s k a et al. (2016) and S l o u p et al. (2016).

Statistical analysis

Zn, Cu, Mn, and Fe concentrations and their statistical differences were compared within groups using the nonparametric Mann-Whitney *U* test. The differences were considered significant at $P < 0.05$. All computations were carried out using the STATISTICA software, Version 10 (Statsoft, USA).

RESULTS

In our study, tapeworm presence decreased element concentrations in the majority of rat tissues (Figs. 1a, b –4a, b). Tapeworms also accumulated higher levels of zinc and manganese than did the majority of host tissues (Fig. 1a, b and Fig. 3a, b). As expected, Zn overdosing significantly increased Zn concentrations in rat testes (Table 1a). In rats with tapeworm infection, zinc levels significantly increased in the testes, spleen, and in the tapeworm of rats with tapeworm infection; however, zinc overdosing surprisingly decreased Zn concentrations in the bone of rats with tapeworm infection (Table 1b).

DISCUSSION

The majority of elements found in humans and animals enter through the oral route and are subsequently absorbed in the digestive tract. This absorption process significantly interferes with gastrointestinal parasites. This is especially true for acanthocephalans and also tapeworms which receive nutrients through the tegument, a metabolically active body surface (S u r e s et al., 2000 a, b, 2002). Tapeworms are able to accumulate considerable amounts of metals, thereby reducing their concentrations in host tissues (J a n k o v s k a et al., 2010 a, b; C a d k o v a et al., 2013; B r o z o v a et al., 2015). Due to a close relationship between the local immune system and epithelial cells in the gastrointestinal tract, local immune reactions

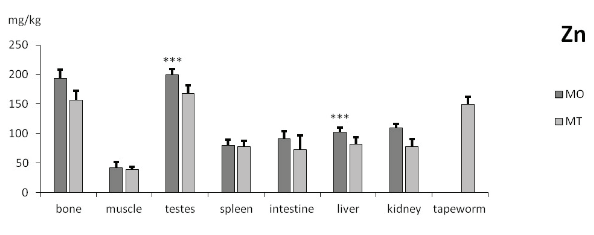

Fig.1a. Zinc concentrations (mg kg–1) in rat tissues and rat tapeworm M0 = rats with zinc lactate in feed mixture, MT = rats with zinc

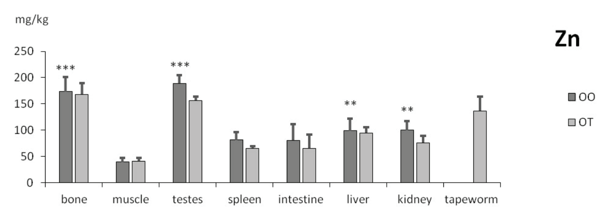

Fig. 1b. Zinc concentrations (mg kg–1) in rat tissues and rat tapeworm 00 = control rats, 0T = control rats with tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

Table 1a. Zn, Cu, Mn, and Fe concentrations (mg kg^{-1}) in rat tissues

Zn	Bone		Muscle		Testes (*)		Spleen		Intestine		Liver		Kidney	
Group/ method	A	M	A	M	A	M	A	M	A	M	A	M	A	M
00	175.3	173.4	42.2	39.4	189.3	189.2	82.8	81.1	82.7	80.6	100.2	98.7	98.5	100.0
M0	193.6	193.2	43.9	41.9	197.6	199.8	77.8	79.6	90.7	91.5	104.2	102.5	103.5	110.0
Cu	bone		muscle		testes		spleen (***)		intestine		liver		kidney	
Group/ method	A	M	A	M	A	M	A	M	A	M	A	M	A	M
00	0.7	0.6	3.3	3.2	11.5	11.3	6.3	6.2	5.1	4.6	13.9	13.4	27.0	27.0
M0	1.0	0.7	3.1	3.0	11.6	11.6	4.1	4.1	5.2	5.0	14.4	14.4	30.5	29.8
Mn	bone (**)		muscle (*)		testes (***)		spleen (***)		intestine (***)		liver		kidney (***)	
Group/ method	A	M	A	M	A	M	A	M	A	M	A	M	A	M
00	0.3	0.3	0.3	0.2	1.7	1.7	0.8	0.8	2.7	2.4	7.3	7.5	3.0	3.1
M0	0.4	0.4	0.3	0.3	2.0	2.0	6.3	5.7	3.3	3.2	7.3	7.3	3.5	3.6
Fe	bone (***)		muscle (**)		testes (***)		spleen		intestine		liver (*)		kidney	
Group/ method	A	M	A	M	A	M	A	M	A	M	A	M	A	M
00	92.52	85.25	68.12	68.62	160.95	161.39	5187.45	4340.49	59.12	59.39	671.45	667.68	351.77	337.23
M0	53.04	54.20	40.40	37.14	132.81	132.62	5103.33	4543.09	50.07	50.20	508.85	517.50	376.46	375.18

M0 = rats with zinc lactate in feed mixture, 00 = control rats, A = arithmetic mean, M = median*weak significance ($P < 0.05$), **medium significance ($P < 0.005$), ***strong significance ($P < 0.0005$)

ll computations were done using program STATISTICA Version 10 (Statsoft, USA)

Table 1b. Zn, Cu, Mn, Fe concentrations (mg kg^{-1}) in rat tissues and rat tapeworm

Zn	Bone (*)		Muscle		Testes (*)		Spleen (*)		Intestine		Liver		Kidney		Tapeworm (*)	
Group/ method	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
0T	167.0	168.4	41.2	41.2	155.0	156.7	68.4	65.6	73.2	65.3	89.0	94.9	83.0	75.9	136.3	136.6
MT	151.7	159.7	39.1	38.6	167.6	168.0	78.0	78.2	77.9	72.5	84.0	82.0	85.1	78.1	158.1	149.2
Cu	bone		muscle		testes (*)		spleen		intestine		liver		kidney		tapeworm	
Group/ method	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
0T	0.9	0.7	3.1	3.1	11.1	11.1	5.4	4.6	5.1	5.0	12.0	12.5	27.1	27.4	5.9	5.1
MT	0.7	0.7	3.2	3.1	10.4	10.5	4.3	4.2	4.8	5.0	12.0	12.2	28.1	29.2	5.6	5.3
Mn	bone		muscle		testes (**)		spleen		intestine		liver		kidney (*)		tapeworm	
Group/ method	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
0T	0.4	0.3	0.2	0.3	1.5	1.5	0.7	0.7	3.8	3.9	5.5	5.4	2.6	2.4	3.4	3.3
MT	0.3	0.3	0.3	0.3	1.4	1.4	0.7	0.7	4.8	4.4	5.4	5.3	2.3	2.3	3.6	3.8
Fe	bone		muscle		testes		spleen		intestine		liver		kidney		tapeworm	
Group/ method	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
0T	67.04	70.43	41.72	39.09	123.23	125.51	4879.61	4855.96	57.98	43.66	446.77	429.19	336.34	327.01	14.34	14.46
MT	69.22	61.40	36.05	32.99	127.60	127.80	3872.92	3428.97	39.50	28.44	496.06	449.02	300.61	273.59	11.78	10.24

MT = rats with zinc lactate in feed mixture and tapeworm infection, 0T = control rats with tapeworm infection, A = arithmetic mean, M = median *weak significance ($P < 0.05$), **medium significance ($P < 0.005$), ***strong significance ($P < 0.0005$)

all computations were done using program STATISTICA Version 10 (Statsoft, USA) can directly alter the epithelial ion transport, causing increased secretion, decreased ion absorption or both (K o s i k - B o g a c k a et al., 2010).

As is evident in Fig 1a, b, tapeworms decreased Zn concentrations in the majority of rat tissues. This phenomenon was significant in the testes and liver tissues in groups with zinc lactate (Fig. 1a). In the control groups, this decrease was significant in the liver, testes, bone, and kidneys (Fig. 1b). This supports the theory regarding the ability of tapeworms to accumulate heavy metals from the host. Hymenolepidiasis is associated with the activation of inflammatory mediators and stimulation of nerve fibres, which significantly affect the function of ion channels in the intestine epithelium of the host (K o s i k - B o g a c k a et al., 2010).

Copper (Cu) is important for proper growth of the body, efficient utilization of iron, proper enzymatic reactions, as well as improved health of connective tissues, hair, and eyes. It is also integral for preventing premature aging and increasing energy production. Apart from these, Cu regulated heart rhythm, balanced thyroid glands, reduced symptoms of arthritis, supported quick wound healing, increased red blood cell formation, and reduced cholesterol (K u c h a r z e w s k i et al., 2003).

Tapeworms significantly decreased Cu concentrations in the testes, liver, and kidneys of rats given overdoses of zinc lactate; surprisingly, Cu concentrations were higher in the spleens of rats with tapeworm infection (Fig. 2a). There were no differences between Cu concentrations in parasitized and unparasitized rats surprisingly, only bone tissues had higher Cu concentrations in rats with tapeworm infection. From these results we can surmise that tapeworm infection has no significant effect on Cu concentrations in host tissues (Fig. 2b). Zn overdosing significantly decreased Cu concentrations in rat spleen (Table 1a) and in the testes of rats with tapeworm infection (Table 1b).

Manganese (Mn) is an essential dietary nutrient and trace element, and at low concentrations it plays an important role in mammalian development, metabolism, and antioxidant defense; however, it becomes neurotoxic at higher concentrations (C h u a , M o r g a n , 1996; M e r c a d a n t e et al., 2016). As we can see in Fig. 3a, b, tapeworms significantly decreased Mn concentrations in the testes, spleen, liver, and

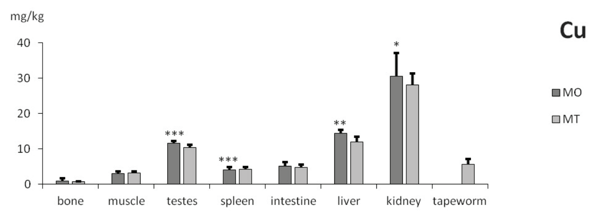

Fig. 2a. Copper concentrations (mg kg–1) in rat tissues and rat tapeworm M0 = rats with zinc lactate in feed mixture, MT = rats with zinc lactate in feed mixture and tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

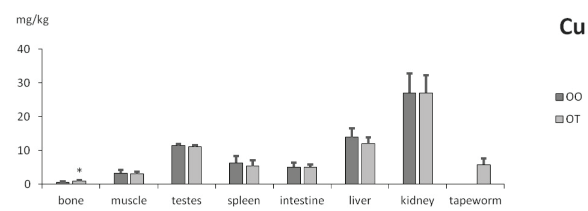

Fig. 2b. Copper concentrations (mg kg–1) in rat tissues and rat tapeworm 00 = control rats, 0T = control rats with tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

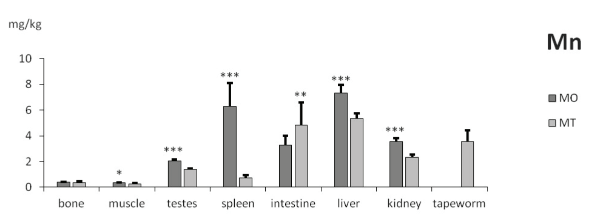

Fig. 3a. Manganese concentrations (mg kg–1) in rat tissues and rat tapeworm M0 = rats with zinc lactate in feed mixture, MT = rats with zinc lactate in feed mixture and tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

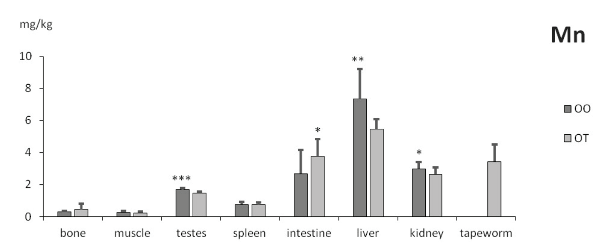

Fig. 3b. Manganese concentrations (mg kg–1) in rat tissues and rat tapeworm 00 = control rats, 0T = control rats with tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

kidneys of rats given overdoses of zinc lactate; interestingly, rats with tapeworm infection had significantly higher Mn concentrations in their intestinal tissues in both groups (Fig. 3a, b). Rats given a Zn overdose had significantly higher Mn concentrations in their spleen, small intestine, kidneys, testes, bone, and muscle than did rats without a Zn overdose (Table 1a). However, rats given a Zn overdose and infected with tapeworms had significantly lower Mn concentrations in their testes and kidneys than did rats with only tapeworm infection (Table 1b).

Iron is a crucial element for both the pathogen/parasite and host in the context of a number of infectious diseases (Navarrete-Perea et al., 2016). In vertebrate hosts, parasites can obtain iron from different host sources including erythrocytes, serum hemoglobin, haptoglobin-hemoglobin complexes, hemopexin, transferrin, and lactoferrin (Cassat, Skaar, 2013).

With respect to iron, tapeworms significantly decreased Fe concentrations in the testicular, bone, muscle and liver tissues of rats not given zinc overdose (Fig. 4b). In rats given a zinc overdose, tapeworms significantly decreased Fe concentrations only in the kidneys. Surprisingly, bone tissues of rats infected with tapeworms and given overdoses of zinc lactate (Fig. 4a) had higher Fe concentrations than those of rats without tapeworm infection. Fe concentrations in the livers of rats not given a Zn overdose (Fig. 4b) were significantly lower in rats with tapeworm infection, i.e., tapeworm infection significantly decreased Fe concentrations in the liver. Zn overdosing in our study prevented significant decreases in Fe concentrations in the livers of rats with tapeworm infection (Fig. 4a). However, there were significantly lower Fe concentrations in the kidneys of rats with tapeworms than in those of rats without tapeworms. This indicates that Zn overdosing did not inhibit the tapeworm's ability to decrease Fe in the kidneys (Fig. 4a). When rats were infected with tapeworms (Table 1b), there were no differences in Fe tissue concentrations between rats given a Zn overdose and those not overdosed with Zn. However, in rats not infected with tapeworms (Table 1a) we found significantly lower Fe concentrations in the bone, muscle, testes, and liver (tissues) of rats given a zinc overdose.

CONCLUSION

Tapeworm presence decreased element concentrations in a majority of rat tissues and tapeworms accumulated more zinc and manganese than did host tissues. Tapeworms can decrease element concentrations in host tissues either through accumulation into their tissues or by increasing intestinal mucus, which decreases ion absorption. Zn overdosing increased Zn concentrations only in the testes, spleen, and tapeworms of rats with tapeworm infection. Moreover, Mn concentrations increased in the spleen, small intestine, kidneys, testes, bone, and muscle of rats given a zinc overdose and not infected with tapeworm. Cu concentrations in rat tissues and the accumulation of Cu, Fe, and Mn by tapeworms were virtually unaffected. Zinc overdosing seems to protect the liver from the absorption of Fe by tapeworms. With the exception of Zn concentrations, element concentrations in tapeworms from hosts given a zinc overdose were similar to those in tapeworms from hosts not given a zinc overdose.

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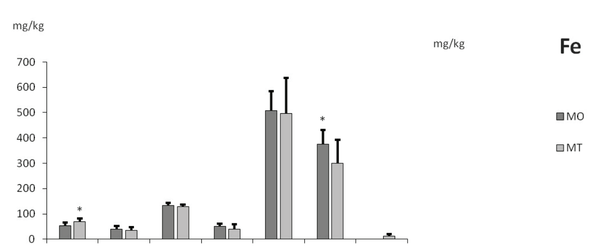

Fig. 4a. Iron concentrations (mg kg–1) in rat tissues and rat tapeworm M0 = rats with zinc lactate in feed mixture, MT = rats with zinc lactate in feed mixture and tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

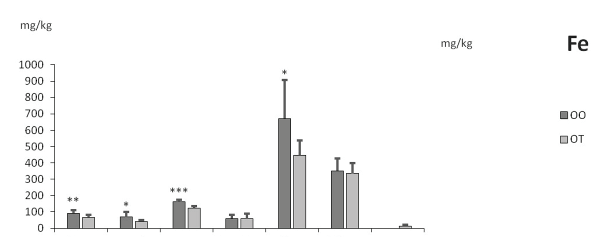

Fig. 4b. Iron concentrations (mg kg–1) in rat tissues and rat tapeworm 00 = control rats, 0T = control rats with tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

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Corresponding Author:

prof. Ing. Ivana J a n k o v s k á , Ph.D., Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Zoology and Fisheries, 165 00 Prague 6-Suchdol, Czech Republic, phone: +420 775 986 602,
e-mail: jankovska@af.czu.cz