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Vliv tasemnice na bioakumulaci zinku a kadmia v těle hostitele

doktorská disertační práce

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Obsah

1 Literární přehled ..... - 1 -
1.1 Kontaminace agroekosystému zinkem a kadmiem ..... - 3 -
1.1 Zinek a kadmium v živočišném těle ..... - 4 -
1.3 Rostliny a rizikové prvky ..... - 16 -
1.4 Tasemnice a rizikové prvky ..... - 20 -
2 Cíl a hypotéza ..... - 23 -
3 Publikované práce ..... - 24 -
3.1 Publikace uvedené v individuálním studijním plánu ..... - 24 -
3.2 Publikace související s tématem disertační práce zveřejněné v časopisech s IF a recenzovaných časopisech ..... - 54 -
3.3 Ostatní publikace ..... - 104 -
3.4 Příspěvky ve sborníku ..... - 148 -
3.5 Dosud nepublikované výsledky ..... - 149 -
4 Diskuze ..... - 151 -
5 Závěr ..... - 160 -
6 Seznam použité literatury ..... - 162 -

1 Literární přehled

Zinek (Zn) je nezbytný pro správný vývoj organismu. Jako stopový prvek u člověka, stejně jako jiných živočichů, je nezbytnou součástí mnoha proteinů, hormonů a enzymů. Podílí se na základních biochemických procesech zajišťujících životní funkce. Mezi nejdůležitější z nich patří proces buněčného dýchání, syntéza DNA a RNA, reprodukce, zachování celistvosti buněčné stěny, je významný antioxidant, odstraňuje volné radikály a ochraňuje buněčné membrány proti peroxidaci lipidů. V lidském těle je obsaženo od 1,5 g do 2,5 g zinku, a to především ve svalovině a kostech (Hotz & Brown 2004; Sun et al. 2006).

Nedostatečné množství zinku je spojeno s širokou škálou vývojových vad, fyziologických anomálií a imunologických problémů. Ty se projevují kožními problémy, jako jsou afty, vyrážky a pomalé hojení ran. Dále se nedostatek zinku projevuje anorexií, ztrátou tělesné hmotnosti, špatným trávením a vstřebáváním potravy, zpomalením a poruchami buněčného dělení, růstovými a neurologickými poruchami, defektním růstem a vývojem kostí. Příčinou jsou změny v aktivitě některých enzymů, hlavně těch, jichž je zinek centrálním atomem (tzv. metaloenzymů), např. ALP, Cu/Zn superoxid dismutázy, laktát dehydrogenázy, karboxypeptidázy a také DNA a RNA polymerázy. Také byla zjištěna zvýšená koncentrace metalothioneinů (proteinů s menší molekulou obsahující aminokyseliny s vázanou sírou, který se syntetizuje v játrech a ledvinách jako reakce na přítomnost dvojmocných kovových iontů) (Kukačka et al. 2008). To je pravděpodobně způsobeno tím, že na úkor zinečnatých kationtů jsou vstřebávány kationty jiných kovů. Metalothionein tyto ionty váže a působí detoxikačně. Zvýšená koncentrace těchto proteinů byla zjištěna u potkanů krmených stravou s nízkou dávkou zinku a stejně tak u potkanů krmených stravou s velmi vysokou dávkou zinku (Cousins 1998; Hotz & Brown 2004; Sun et al. 2006).

Zinek je zapojen v zachování a údržbě střevních struktur a funkcí. Jeho přítomnost podporuje imunitu ve střevech a tím udržení správné rovnováhy rozložení mikrobiologického prostředí. Doplnění nedostatku zinku vede k urychlení regenerace střevní sliznice a zvýšení produkce enzymů kartáčového lemu. Adekvátní množství zinku ve stravě zlepšuje trávení a aktivitu hydroláz ve střevech. Nedostatek zinku

způsobuje kratší, scvrklejší a více zploštělé klky v jejunu. Bylo zjištěno, že důsledkem je značně snížené vstřebaní stravy na plochu střevní stěny oproti zdravému jejunu (Hotz & Brown 2004).

Předávkování zinkem běžnou stravou není možné, snad jen v případě nadměrné konzumace některých druhů mořských ryb, podávání doplňků výživy obohacených zinkem ve vysokých dávkách či dlouhodobým používáním vody přiváděné pozinkovaným potrubím. V takových případech může mít předávkování zinkem za následek narušení metabolismu jiných kovů. Pokud je zinek přijímán ve velmi vysokých dávkách, je ve střevech vstřebáván na úkor jiných kovových iontů a snižuje se tak přijaté množství mědi, železa, kobaltu nebo chromu. Trvá-li tento stav dlouhodobě, mohou se projevit např. příznaky anémie. Z toho je patrné, jak je důležité dbát na vyvážený příjem všech biogenních kovů. Hlášeno bylo jen velmi málo případů, kdy došlo k akutní otravě zinkem. V těchto případech postižení trpěli nevolností, zvracením, průjmem, horečkou a letargií (WHO 1996; Hotz & Brown 2004; Kukačka et al. 2008).

Kadmium je pro živočišný organismus oproti zinku kov výhradně toxický. Toxicita kadmia závisí na celé řadě faktorů, jako je množství denního příjmu, forma přijímaného kadmia a celkový stav jedince (Chmielnicka & Cherian 1986).

Kadmium je akumulováno především v ledvinách, a to i při velmi nízké expozici. Pokud je jedinec kadmiu vystaven chronicky, či pokud je jednorázové množství vysoké, může dojít k poškození ledvin, v jehož důsledku mohou ledviny přestat plnit svou funkci. Příjem kadmia se však projevuje negativně také v ostatních tkáních, kdy způsobuje řadu poruch, jako je osteoporóza, která se může rozvinout do své závažnější podoby osteomalacie a projevit se může onemocnění itai-itai (Zheng et al. 2014).

Vzhledem k velké podobnosti z hlediska chemických a fyzikálních vlastností mezi zinkem a kadmiem, dochází v případě intoxikace organismu kadmiem ke kompetici mezi zinkem a kadmiem. V případě nahrazení zinku toxickým kadmiem v enzymech či hormonech, dochází ke ztrátě jejich funkce a tím k narušení správného průběhu životně důležitých metabolických procesů v organismu. Kadmium ovlivňuje buněčnou proliferaci, diferenciaci a apoptózu. To souvisí s mechanismem opravy DNA, generováním reakčních forem kyslíku (ROS) a indukcí apoptózy. Kadmium se váže na mitochondrie a může inhibovat jak buněčné dýchání, tak oxidativní

fosforylaci, a to i při velmi nízké koncentraci. Kadmium také negativně ovlivňuje příjem a vazbu jiných pro život nezbytných mikroprvků, jako je měď, kobalt, mangan, železo a selen (Abdulla & Chmielnicka 1989). Rovněž bylo kadmium klasifikováno jako karcinogen, mutagen a teratogen. Velmi negativně ovlivňuje nervový systém (Winiarska-Mieczan & Kwiecień 2016).

Vzhledem k tomu, že jak Zn, tak Cd jsou stejně jako většina kovů u živočichů přijímány hlavně perorální cestou a následně vstřebávány v trávicím ústrojí, zasahují do tohoto procesu vstřebávání významným způsobem gastrointestinální parazité, včetně tasemnic (Sures et al. 2002, 1999; Eira et al. 2005; Kosik – Bogacka et al. 2010).

Infekce trávicího traktu parazity vyvolává kromě patofyziologických změn také imunologickou odpověď u hostitele. Vzhledem k úzkému vztahu mezi epitelovou buňkou trávicího traktu a imunitním systémem, mohou imunitní reakce v důsledku přítomnosti tasemnice ovlivnit transport iontů v epitelu střeva, což může mít za následek zvýšenou sekreci, snížení vstřebáváni iontů nebo obojí. Infekce tasemnicí má tudíž značný vliv na přenos iontů těžkých kovů do krevního řečiště (Kosik – Bogacka et al. 2010).

Tasemnice rodu Hymenolepis jsou navíc schopné akumulovat značná množství kovových iontů přijatých hostitelem (Sures et al 2002, 2003; Eira et al. 2005; Sun et. al. 2006).

1.1 Kontaminace agroekosystému zinkem a kadmiem

Zinek a kadmium vstupují do agroekosystémů dvěma způsoby. Prvním se do půdy dostávají během zvětrávání matečné horniny, ve které jsou obsažené. Tři hlavní zinek obsahující minerály jsou smithsonit ($ZnCO_3$), sfalerit (ZnS) a hemimorfit $Zn_4Si_2O_7(OH)_2•H_2O$. Minerály obsahující kadmium jsou andyrobertsit ($KCdCu_5(AsO_4)_4[As(OH)_2O_2]·H_2O$) a hawleyit (CdS). Tyto minerály mohou být pod vlivem specifických podmínek v životním prostředí rychle zvětrávány, přičemž zinek a kadmium v nich obsažené mohou být oxidovány a tím se stát mobilními.

Druhým způsobem je antropogenní činnost. Antropogenní procesy zahrnují aplikace hnojiv, fungicidů, herbicidů, pesticidů a rybničních sedimentů na zemědělskou půdu, která obsahují značná množství různých stopových kovů

včetně zinku. Nevhodné nakládání s průmyslovým a komunálním odpadem, nebo průmyslové imise jsou zase častým zdrojem kadmia.

Mobilita a dostupnost stopových prvků je ovlivněna řadou chemických a biochemických procesů, jako je rozpouštění ve srážkové vodě, adsorpce-desorpce, komplexotvorné reakce, disociace a oxidačně – redukční reakce. Mobilitu zinku a kadmia také ovlivňuje pH půdy a různé biologické procesy (Zhenli et. al., 2005).

1.1 Zinek a kadmium v živočišném těle

Vzhledem ke svým fyzikálněchemickým vlastnostem se zinek podílí na široké škále katalytických, strukturních a regulačních buněčných funkcí. Plní roli biokatalyzátoru, transportéru a detoxikantu (Kukačka et al. 2008; Erdman et al. 2012).

Zinek je druhým nejrozšířenějším stopovým prvkem v těle. Celková koncentrace zinku v savčích buňkách se odhaduje na 100 až 500 μM. Většina iontů $Zn2^{+}$ je pevně vázána různými proteiny, které hrají důležitou strukturální a/nebo katalytickou roli v transkripčních faktorech a v řadě sekrečních, na membráně vázaných a také endozomových/lysozomových enzymů (Petkovic et. 2013).

Více než 300 enzymů potřebuje zinek pro své katalytické funkce. Pokud je zinek z katalytického místa odstraněn, enzym přestane plnit svou funkci. Na rozdíl od jiných kovů, enzymy jejichž součástí je zinek se nachází ve všech šesti enzymatických třídách (oxidoreduktázy, transferázy, hydrolázy, isomerázy, lyázy a lygázy). Zinek slouží jako akceptor elektronů a tím umožňuje aktivitu enzymů. Apoptóza neboli programovaná buněčná smrt, je rovněž regulována zinkem. Zinek se podílí i na zdvojení DNA a transkripci RNA. Nezbytný je pro správnou funkci řady hormonů, například pro růstový hormon, pohlavní hormony, glukagon a dále je zinek nepostradatelný při syntéze a působení insulinu. Insulin je skladován v sekrečních měchýřcích buněk Langerhansových ostrůvků pankreatu, kde je uspořádán v pravidelné krystalické struktuře zahrnující ionty zinku. Každá molekula insulinu je spojená s 2 až 4 atomy zinku. Zinek/insulin komplex je vytvořen za účelem pomalého uvolňování insulinu do krevního řečiště, kam je vyplavován průběžně a jeho hladina stoupá při zvýšené hladině krevní glukózy (Hotz & Brown 2004).

Zinek hraje v metabolismu bílkovin a nukleových kyselin nepostradatelnou úlohu, kdy přibližně 10 % proteinů kódovaných v savčím genomu je na zinku

z hlediska struktury a funkce závislých. Zinek je nepostradatelný pro funkci buněčných membrán, je zapojen do tvorby pojivové tkáně, zubů, nehtů, vlasů (srsti) a je důležitou součástí metabolismu vápníku (Erdman et al. 2012).

Zinek nacházející se v játrech, kostře, plazmě a pankreatu je rychle dostupný pro metabolické procesy. Za situace, kdy v krmivu je zinku nedostatek, se ze zmíněných tkání zinek vyloučí do krve, přičemž koncentrace v mozku a svalech se nemění. Metabolismus zinku mohou negativně ovlivnit látky jako chelatační činidla a některá antibiotika, např. penicilin (Kafka & Punčochářová 2002).

Kadmium patří mezi prvky, jejichž vliv na zdravotní stav lidského organismu je výhradně negativní. Tento fakt se zdá být zajímavý mimo jiné proto, že kadmium je chemicky velmi podobné zinku. Právě vzájemná chemická podobnost těchto prvků působí problémy, protože kadmium může snadno vstupovat do různých enzymatických reakcí místo zinku a následné životně důležité biochemické pochody neproběhnou nebo probíhají jiným způsobem. Příkladem je zablokování inzulínového cyklu, které může působit vážné zdravotní komplikace. Akumulace kadmia v prostatě, kde je běžně vysoký obsah zinku, může být příčinou rakoviny prostaty (Brzóska & Moniuszko-Jakoniuk 2001). Kadmium je mimořádně kumulativní toxický prvek. Přijaté kadmium se z organismu vylučuje velmi pozvolna, přičemž většina se ukládá především v ledvinách a v menší míře také v játrech. Kadmium může v ledvinách setrvat až desítky let. Právě ledviny jsou tak při otravě kadmiem nejvíce ohroženy i z dlouhodobého hlediska (Brzóska & Moniuszko-Jakoniuk 2001).

Četné studie prokázaly, že zinek může snížit karcinogenitu a celkovou toxicitu kadmia snížením absorpce kadmia. Toxické hladiny kadmia však naopak mohou inhibovat absorpci zinku. Studie provedené na buňkách ledvin a tenkého střeva ukazují, že zinek a kadmium mohou sdílet transportní a vazebné mechanismy při transportu přes epitel. V nízkých koncentracích působí kadmium jako kompetitivní inhibitor vychytávání zinku, zatímco ve vyšších koncentracích vykazuje nekompetitivní inhibici (Brzoska et al. 2001).

Zdroje zinku

Potrava představuje hlavní zdroj zinku. Doporučený denní příjem zinku je přibližně 15 mg, přičemž u mužů a těhotných žen může být doporučené denní množství o něco vyšší s maximem 20 mg/den. Poměrně vysoká koncentrace Zn je především v mase (zejména hovězí, vepřové, ale i krůtí a kuřecí), mořských produktech (např. ústřice jsou považovány za nejbohatší zdroj zinku), ale také obilovinách a luštěninách. Obecně se lze říct, že výhodnějšími zdroji zinku jsou živočišné potraviny oproti rostlinným. Živočišné potraviny neobsahují téměř žádné sloučeniny, které by inhibovaly absorpci zinku, důležitý je zejména nulový obsah fytátů. Kyselina fytová snižuje biologickou dostupnost Zn tak, že s ním tvoří nerozpustné komplexy. Kdežto přítomnost určitých aminokyselin, jako je cystein a histidin, absorpci zinku zlepšují (Cousins 1998; Brown et al. 2001; Erdman et al. 2012).

Molární poměr mezi kyselinou fytovou a zinkem je určitým měřítkem biologické využitelnosti zinku. Světová zdravotnická organizace (WHO) definovala 3 kategorie potravin s vysokou, střední a nízkou biologickou dostupností zinku (Brown et al. 2001).

Kategorie A - potraviny, které neobsahují žádné známé inhibitory absorpce zinku, využitelnost zinku je vysoká (molární poměr fytát : Zn je do 5)

Kategorie B - potraviny, které obsahují malé množství inhibitorů absorpce Zn (molární poměr fytát : Zn je v rozmezí 5 - 15)

Kategorie C – potraviny, které obsahují velké množství inhibitorů absorpce Zn (molární poměr fytát : Zn je nad 15)

Suplementy zinku

Zabránit nedostatečnému množství přijímaného zinku organismem lze předejít obohacováním potravy o suplementy zinku, tedy chemické sloučeniny nezávadné pro zdraví obsahující zinek.

Takových sloučenin existuje celá řada. Liší se výrobní cenou a rovněž biologickou dostupností pro organismus, tedy rozdílnou mírou absorpce v trávicím traktu. Oxid zinečnatý a uhličitan zinečnatý jsou nejběžněji používané suplementy.

Jejich nevýhodou je horší rozpustnost ve vodě a tím omezená vstřebatelnost v gastrointestinálním traktu. Allen (1998) popsal nízké plasmatické koncentrace Zn po příjmu stravy obohacené o oxid zinečnatý a uhličitan zinečnatý ve srovnání s hladinami zinku v plasmě po přijetí stravy obohacené o soli octanu zinečnatého a síranu zinečnatého. Příjem zinku ve formě potravinových suplementů by neměl překročit 20 mg/den u zdravých dospělých osob, ačkoliv Národní výzkumná rada Národní akademie věd USA stanovila tolerovatelnou horní hranici 40 mg/den (Maret & Sandstead 2006).

Chronické předávkování Zn, v rozmezí 100–300 mg/den u dospělých osob způsobit nedostatečný příjem mědi (Prasad et al. 1998) a tím změny v imunitní odpovědi a hladině sérových lipoproteinů. Tyto poruchy se mohou objevit i při nižších dávkách (50 mg Zn/den) (Plum et al. 2010).

Mléčnan zinečnatý

Biologicky dobře dostupným suplementem je mléčnan zinečnatý. Mléčnan zinečnatý se řadí v České republice podle Přílohy č. 2 k vyhlášce č. 446/2004 Sb. mezi sloučeniny zinku užívané jako doplňky stravy. Podle uvedené vyhlášky je nejvyšší přípustné množství zinku pro člověka v denní dávce 25 mg a doporučené denní množství je 15 mg.

Biologickou dostupnost laktátu (mléčnanu) zinečnatého a glukonátu zinečnatého porovnávali ve své studii Shengkui et al. (1994). Autoři krmili potkany sójovou potravou s nedostatkem zinku. Po měsíci nedostatečného množství zinku v potravě podali potkanům potravu obohacenou o mléčnan zinečnatý a o glukonát zinečnatý. Po čtyřech týdnech změřili potkanům hladinu zinku v krevním séru. Bylo zjištěno, že mléčnan zinečnatý vykazuje vyšší biologickou dostupnost, což autoři připisují je menší molekulové hmotnosti. Vzhledem k tomu, že podle studie Allen (1998) byl glukonát zinečnatý vyhodnocen jako biologicky dostupnější spolu s octanem zinečnatým ve srovnání se síranem zinečnatým a uhličitanem zinečnatým a vzhledem k tomu, že mléčnan zinečnatý dle studie Shengkui et al. (1994) byl vyhodnocen jako biologicky dostupnější ve srovnání s glukonátem zinečnatým, zdá se, že mléčnan zinečnatý je z těchto pěti potravinových suplementů zinku biologicky nejdostupnější.

Homeostáza zinku

Neustálý proces udržení rovnováhy mezi příjmem zinku v potravě a jeho vylučováním, tedy snaha o udržení stabilní hodnoty zinku v organismu, se nazývá homeostáza zinku (Erdman et al. 2012). V tomto procesu hrají nejvýznamnější roli gastrointestinální systém (hlavně tenké střevo), slinivka břišní, játra a ledviny. Procesy zapojené do tohoto procesu jsou především absorpce exogenního zinku, gastrointestinální sekrece, vylučování endogenního zinku, zadržení zinku ve tkáních nebo snížené/zvýšené vylučování močí (Krebs 2000).

Zdroje kadmia

Zdroje kontaminace kadmiem souvisí s jeho použitím v průmyslu jako antikorozního činidla, jeho použití jakožto stabilizátoru ve výrobcích z PVC, jako součást pigmentů barev a v Ni-Cd bateriích. Antropogenní zdroje kadmia v životním prostředí pocházejí ze zpracování rud jiných kovů, především při získávání mědi a niklu, ze spalování fosilních paliv a používání fosfátových hnojiv. Kadmium je také přítomno jako znečišťující látka v hutích neželezných kovů a při recyklaci elektronického odpadu. Sopečná činnost, eroze a abraze hornin a půdy a lesní požáry patří mezi další příčiny nárůstu koncentrací Cd v životním prostředí (Rafati-Rahimzadeh et al. 2017; Genchi et al. 2020).

Kadmium může být v kontaminovaných oblastech ve značném množství absorbováno z vody, potravy a prachu rozptýleného ve vzduchu. Vysoké koncentrace Cd nacházíme v korýších, mlžích, ústřicích, hlavonožcích a krabech a produktech z vnitřností, především v těch, jejichž součástí jsou játra a ledviny. Dále v rostlinných produktech, jako jsou olejnatá semena, kakao, fazole a některé volně rostoucí houby. Potraviny získané z rostlin rostoucích na půdě s nadměrnou koncentrací kadmia, obsahují obecně vyšší množství Cd než živočišné produkty jako maso, vejce, mléko a mléčné výrobky získané od zvířat vystavených vyšším dávkám kadmia. Především rýže, pšenice, zelená listová zelenina, brambory, mrkev a celer mohou obsahovat vysoké dávky kadmia (Rafati-Rahimzadeh et al. 2017; Genchi et al. 2020).

Absorpce zinku a kadmia

Absorpce zinku i kadmia z přijaté potravy probíhá pomocí enterocytů, přes bazolaterální membránu a následné dopravení kovu do portálního oběhu (Cousins et al. 2006). Z potravy se oba prvky uvolňují v podobě volných iontů. K tomu dochází převážně v oblasti distálního duodena a proximálního jejuna. Asi 60 % absorpce probíhá v duodenu, 30 % v ileu, 8 % v jejunu a 3 % ve slepém a tlustém střevě. Zinek i kadmium jsou absorbovány dvěma mechanismy, aktivním a pasivním transportem (Hotz & Brown 2004).

Zinek podávaný ve vodných roztocích nalačno se vstřebává efektivně (60–70 %), zatímco absorpce z pevné stravy je méně účinná a liší se v závislosti na obsahu zinku a složení stravy. Obecně je 33 % přijímáno jako průměrná absorpce zinku u lidí (Cousins et al. 2006).

Kadmium přijímané potravou se vstřebává jen zhruba z jednoho až pěti procent, zatímco kadmium ve formě aerosolu či jemného prachu ve vzduchu je v dýchacích cestách vstřebáváno i z více než padesáti procent (Brzóska & Moniuszko-Jakoniuk 2001).

Aktivní transport obou kovových prvků je uskutečňován pomocí specifických přenašečů. Účinnost se zvyšuje při nižším množství zinku v potravě. Pasivní transport funguje na principu difúze a jeho rychlost je přímo úměrná koncentraci zinku ve střevě (Krebs 2000).

Distribuce zinku a kadmia

Absorbovaný zinek a kadmium jsou v krevní plasmě transportovány nejčastěji ve vazbě na albumin (60–80 %). V menším rozsahu na α-2-makroglobulin a transferin. Také jsou vázány na volné aminokyseliny, a to především na histidin a cystein. Vazba na plasmatické bílkoviny představuje nejdůležitější volnou a snadno dostupnou zásobu zinku v organismu, ačkoliv tento Zn představuje jen okolo 0,1 % celkového množství zinku v těle (Cousins et al. 2006).

Kovy transportované do jater jsou odsud dále uvolňovány do celého těla. V hepatocytu, stejně jako v enterocytu a dalších buňkách, jsou Zn a Cd uchovávány ve vazbě na metaloproteiny. Ty zahrnují metaloenzymy, zásobní proteiny a přenašeče. V hepatocytu jsou vázány na metalothionein (Cousins et al. 2006).

Zinkové metaloproteiny

Proteiny závislé na zinku jsou nejpočetnější skupinou metaloproteinů v lidském těle. Struktura těchto proteinů je rozličná a v těle plní širokou škálu funkcí – od biokatalyzátorů, přes transportéry, detoxidanty až po transkripční faktory. Zinkové metaloproteiny je možno formálně rozdělit na zinkové enzymy a zinkové neenzymové proteiny. Mezi zinkové neenzymové proteiny řadíme proteiny pro distribuci zinku (ZnT a ZIP), zinkové prsty a metalothioneiny (Kukačka et al. 2008).

Zinkové prsty

Označení zinkové prsty je používáno pro strukturní motiv, který nacházíme u celé řady bílkovin. Pojem zinkové prsty vznikl podle tvaru, který vytváří při specifické posloupnosti zhruba 30 aminokyselin s pevnou vazbou k zinečnatému atomu. Zinkové prsty, respektive tento strukturní motiv, umožňuje vazbu určitých bílkovin na DNA a RNA. Je proto součástí řady transkripčních faktorů. Uvnitř zinkového prstu se nachází vazebné místo pro zinek koordinačně navázaný na cysteinový a histidinový zbytek (Hartwig 2004).

Proteiny, které obsahují zinkové prsty, jsou významné např. při rozvoji epitelu, organizaci cytoskeletu, transkripci, translaci, buněčné adhezi a uspořádání proteinů do terciálních struktur. Struktury zinkových prstů se často nacházejí v transkripčních faktorech a opravných proteinech DNA, zprostředkovávajících vazbu DNA-protein a protein-protein. Ukázalo se, že některé toxické kovové prvky, včetně kadmia, interferují s transkripcí a opravou DNA. Kadmium způsobuje vytěsnění zinku a tím tvorbu smíšených komplexů a oxidaci cysteinových zbytků v doméně vázající kov. Nesprávné skládání domén se zinkovým prstem způsobené přítomností kadmia je spojeno se ztrátou funkce proteinu. Narušení struktury zinkových prstů tak může vést k nesprávné funkci mnoha buněčných procesů zapojených do genové exprese, regulace růstu a udržování genomové integrity (Hartwig 2004).

Proteiny pro transport zinku

Transportéry zinku rozdělujeme do dvou skupin – skupina ZnT a skupina ZIP. Transportéry skupiny ZnT exportují zinek z cytoplazmy. Jsou nalézány především na

Golgiho aparátu, cytoplazmatické membráně, endoplazmatickém retikulu a endozomech. Tato skupina zahrnuje ZnT-1, který byl lokalizován na plazmatické membráně, kde slouží jako exportér zinku z buňky do extracelulárního prostoru téměř ve všech tkáních. Dále ZnT-2 funguje také jako exportér Zn z buňky ven, má ale ještě schopnost transportovat zinek do vesikulů za podmínek vysoké koncentrace zinku v buňce. Tuto funkci plní hlavně v acinárních buňkách pankreatu. Dále se nachází ve střevě, ledvinách a varlatech. Aktivita ZnT-3 je spojována s přenosem zinku do vesikulů a jeho exprese je omezená na mozek, což nasvědčuje důležité roli zinku v centrálním nervovém systému. ZnT-4 je přepisován ve žlázách savců a v mozku. ZnT-5 je lokalizován na vesikulech sekrečních buněk pankreatu a na apikální membráně enterocytů (ta část plazmatické membrány, která je specializována pro absorpci – kartáčový lem). ZnT-10 je lokalizován na plazmatické membráně (Cousins et al. 2006; Coyle et al. 2002; Huang et al. 2005; Kukačka et al. 2008; Robinson et al. 2001).

Druhou skupinu transportérů pod označením ZIP, rozdělujeme do 4 podskupin. Tyto transportéry transportují zinek do cytoplasmy buněk jednak z extracelulárního prostředí nebo z vesikulů. Většina jich je lokalizována na cytoplazmatické membráně, kromě ZIP-7, který se nachází na Golgiho aparátu. ZIP-14 se nachází na membráně v hepatocytech. V případě akutního zánětu zvyšuje absorpci zinku (Coyle et al. 2002; Kukačka et al. 2008; Robinson et al. 2001).

Metalothioneiny

Skupina intracelulárních proteinů s nízkou molekulovou hmotností, které jsou schopny vázat dvojmocné kationty kovů, včetně zinku a kadmia. Metalothionein (MT) je složen z 60–68 aminokyselin, dvacet z těchto aminokyselin je pouze cystein a nevyskytuje se zde žádná aromatická aminokyselina ani histidin. V řetězci aminokyselin jsou zastoupeny motivy Cys-Cys, Cys-X-Cys a Cys-X-X-Cys (X značí jinou aminokyselinu než cystein). Lidský genom obsahuje minimálně 16 genů pro MT. Ty kódují proteiny s úzce příbuznými sekvencemi a jsou exprimovány v různých typech tkání – nejčastěji v játrech, ledvinách, střevě, slinivce a mozku (Coyle et al. 2002; Robinson et al. 2001).

Pro všechny MT je charakteristický nejen vysoký obsah cysteinu, ale i vazba kovových iontů pomocí thiolátů a tvorba cysteinyl – thiolátových klastrů s charakteristickým prostorovým uspořádáním (Coyle et al. 2002; Robinson et al. 2001).

Metalothionein plní v organismu celou řadu funkcí. Nejvýznamnější funkcí je transport esenciálních iontů kovů a detoxikace toxických hladin iontů kovů. Nejčastěji váže právě zinek a v případě intoxikace rovněž kadmium. Plní také funkci zásobárny přebytečných kovových iontů, která může být využita v době nedostatečného příjmu iontů kovů (Miles et al. 2000).

Zinkové enzymy

Metaloenzymy jsou enzymatické proteiny obsahující ionty kovů (kovové kofaktory), které jsou přímo vázány na protein. Asi jedna třetina všech známých enzymů jsou metaloenzymy (Hoppert 2011). Zastoupeny jsou ve všech šesti enzymových třídách, kde v enzymech závislých na zinku zastávají tři důležité funkce – katalytickou, ko-katalytickou a strukturní. Tam, kde se zinek v enzymech podílí na katalytických procesech, je definován jako Lewisova kyselina – přijímá elektron (karboanhydráza, alkoholdehydrogenáza). Atom zinku je v aktivním místě řízen třemi aminokyselinami, nejčastěji histidinem, a jednou molekulou vody. Zinek může mít v enzymech také ko-katalytickou funkci, zde jsou 2 nebo 3 atomy zinku. Tam, kde zinek zastává strukturní funkci, je nejčastěji vázán 4 aminokyselinovými zbytky uvnitř proteinu a zajišťuje jeho aktivitu (Hotz & Brown 2004).

Matrixové metaloproteinázy

Matrixové metaloproteinázy (MMP) jsou endopeptidázy vyžadující pro svou funkci zinek. Jsou secernovány z buněk v neaktivní formě. Aktivovány jsou např. plasminem a podílejí se na štěpení bílkovin extracelulární matrix např. kolagenu a lamininu. Proteolytická aktivita MMP je inhibována nespecifickými inhibitory alfa2-makroglobulinem a α1-antiproteázou a rovněž specifickými inhibitory (tkáňovými inhibitory metaloproteináz – TIMP). Mezi MMP patří kolagenáza a gelatináza. Důležitou funkci mají při regeneraci tkání, hojení a tvorbě jizvy či zánětu (Ogata et al. 2001).

MMP jsou tvořeny signálním peptidem, propeptidem, katalytickou doménou obsahující místo pro zinek a vápenaté ionty, otáčivou oblast a hemopexinovou doménu. Katalytická doména udržuje trojrozměrnou strukturu MMP (Ogata et al. 2001).

Aktivace MMP závisí na cysteinovém spínači. Ten je tvořen vazbou cysteinu v propeptidu se zinkem v katalytickém místě. Vytvořením vazby mezi cysteinem a Zn^{2+} dochází k zablokování aktivního místa enzymu. To znemožní navázání substrátu, který je poté štěpen. Rozpojením této vazby dochází k aktivaci enzymu. Molekula vody se následně naváže na Zn^{2+} a po disociaci nahrazuje cysteinový zbytek. V organismu je propeptid odštěpen pomocí proteáz (nejčastěji plazminem) nebo oxidativním stresem (Ogata et al. 2001)

Imunitní systém

Zinek hraje velmi důležitou roli v imunitním systému a jedinci s nedostatečným příjmem zinku jsou ohroženi sníženou odolností vůči různým patogenům. Zinek ovlivňuje imunitní systém v mnoha směrech, od základu imunity jako je kožní bariéra až po genovou regulaci v lymfocytech. Zinek je rozhodující pro normální vývoj a funkci buněk zajišťujících první rychlou obrannou linii při zásahu těla patogeny, tedy buněk, jež se podílejí na nespecifické imunitě, jako jsou neutrofily a NK buňky. Zinek také ovlivňuje vývoj získané imunity tím, že jeho nedostatek brání jak růstu, tak některým funkcím T-lymfocytů, např. aktivaci a produkci cytokinů. Také má vliv na funkci B-lymfocytů, kdy je omezen jejich vývoj a produkce protilátek, zejména imunoglobulinu G. Makrofág, buňka, jež je klíčová v mnoha imunologických funkcích, je negativně ovlivněna nedostatkem zinku. Tím je ohrožena produkce cytokinů a proces fagocytózy. Účinky zinku na tyto klíčové součásti imunitní odpovědi se odvíjí od mnoha funkcí zinku v základních buněčných procesech, jako je replikace DNA, transkripce RNA a buněčné dělení. Zinek také plní roli antioxidantu a dokáže stabilizovat membrány (Caballero et al. 2005).

Reprodukce

Zinek je nezbytný také v procesu reprodukce. Snížený příjem je doprovázen opožděným pohlavním vývojem a hypogonadismem, tedy nesprávnou funkcí

samčích pohlavních žláz spojenou s nízkou hladinou testosteronu a sníženou tvorbu spermií. Zinek se podílí i na motilitě a penetraci spermií. Také se podílí na tvorbě a zrání spermií při spermatogenezi, na růstu varlat, syntéze steroidních hormonů, syntéze stimulujícího hormonu (FSH) a také luteinizačního hormonu (LH). Zinkové prsty slouží jako mediátor při biologickém účinku estrogenů a androgenů. Studie na potkanech zjistily výrazně menší Leydigovy buňky při nedostatku zinku oproti zvířatům s optimální hladinou zinku. U samic se zinek účastní syntézy prostaglandinů a kyseliny arachidonové. Ovlivňuje proces vylučování prolaktinu z předního laloku hypofýzy. U březích samic má nedostatek zinku za následek zpomalení vývoje plodu, kongenitální malformace, nepravidelné a těžké porody, sníženou laktaci a nízkou životaschopnost narozených mláďat (Zeman et al. 2006; Salgueiro 2000).

Deficit zinku

Nedostatek zinku je možné hledat v řade obecných příčin, jež mohou nedostatek tohoto esenciálního kovu způsobit buď individuálně, nebo se jejich efekt může sečíst. Patří mezi ně především nedostatečný příjem, zvýšená potřeba, malabsorpce, vysoká ztráta a zhoršené využití. Jednoznačně nejběžnější příčinou je nedostatečný příjem zinku ve stravě způsobený buď nízkým celkovým příjem stravy, konzumací stravy primárně s nízkým obsahem zinku obecně anebo konzumací stravy se zinkem, který je špatně vstřebatelný. Nízký příjem zinku ve stravě je poměrně rozšířený fakt bez vazby na konkrétní geografickou oblast nebo místní kulturní a stravovací zvyklosti (Hotz & Brown 2004).

Nedostatek zinku v organismu může být také způsoben stavem jedince, tedy fyziologickými nebo patologickými změnami, které vedou k vyšším nárokům na množství přijatého zinku. Malabsorpce zinku může být dalším důvodem nedostatku zinku v organismu a může být způsobena řadou různých faktorů. *Acrodermatitis enteropathica* je vzácné kožní onemocnění spojené s nedostatkem zinku. Malabsorpční syndrom či zánětlivé onemocnění střev, mohou mít za následek špatné vstřebávání a/nebo ztráty zinku z těla a mohou urychlit vznik nedostatku zinku v těle, zejména pokud příjem zinku v potravě není dostatečný. Některé léky, jako je fenytoin nebo tetracyklin, také snižují absorpci zinku. Rovněž vysoký příjem jiných biogenních kovových prvků ve vyšším množství, nejčastěji je toto riziko spojeno s vysokými

dávkami železa v potravě, může být příčinou nízké hladiny zinku v organismu. Dalšími důvody deficitu zinku mohou být růst a dospívání, těhotenství a kojení, rekonvalescence, pooperační stavy, silné průjmy, popáleniny a nádorové onemocnění (Salgueiro 2000).

Exkrece zinku a kadmia

Hlavní cestou exkrece zinku a kadmia je gastrointestinální trakt, kde hlavním zdrojem jsou pankreatické šťávy, žluč, gastroduodenální sekrety a transepiteliální přestup z buněk mukózy. Velká část zinku je znovu resorbována ze střeva, čímž je udržována jeho dostatečná hladina v těle při různém příjmu. Množství endogenně vyloučeného zinku se pohybuje od 0,5 do 3 mg/den, a to především v závislosti na celkovém příjmu zinku (Erdman et al. 2012). Střevní a pankreatická recirkulace zinku je velmi důležitá pro udržení jeho správné homeostázy.

Řada studií zabývajících se exkrecí zinku udává, že 90–98 % kovu je vylučováno stolicí. Ta obsahuje 1–5 mg Zn/24 h. Menší část Zn (2–10 %) je vylučována močí. Dále se Zn ztrácí z těla ve vlasech, potu a v odloupaných kožních epiteliích. V laktaci je Zn vylučován rovněž mlékem. To je dáno tím, že 95 % zinku filtrovaného v glomerulu se resorbuje. Ačkoli je exkrece zinku močí relativně nízká, pomáhá udržovat homeostázu při extrémním příjmu zinku. K velkým ztrátám zinku močí dochází v případě svalového katabolismu, v případech těžkých popálenin, traumatech, hladovění, při léčbě chelatačními činidly a při onemocnění jater. Svalový anabolismus naopak snižuje výdej zinku močí (Erdman et al. 2012; Krebs 2000). Také alkohol zvyšuje vylučování zinku močí, přičemž snižuje jeho koncentraci v krvi a plazmě (Zeman et al. 2006).

Zinek vyloučený potem, vlasy, spermatem a odlupováním buněk epitelu může představovat až 17 % celkového vyloučeného zinku. V době růstu nebo laktace se vylučování zinku močí snižuje (Hotz & Brown 2004).

K odstranění těžkých kovů jsou v játrech využívány metalothioneiny, na cystein bohaté proteiny s vysokou afinitou k dvojmocným kovovým iontům jako Cd^{2+}, Cu^{2+}, Hg^{2+} a Zn^{2+}. Tyto kovové ionty zahajují tvorbu metalothioneinů pomocí zvláštního kovy regulujícího elementu (MRE) v genovém promotoru (Koolman & Röhm 2012).

1.3 Rostliny a rizikové prvky

Kovy jsou v půdě navázány na půdní částice, jsou tak pro rostliny nedostupné. Vzhledem k tomu, že rostliny mohou přijímat jen volné ionty, tak kořeny do rhizosféry uvolňují cheláty a kationty H^+. Tyto kořenové exudáty ovlivňují rozpustnost prvků a jejich příjem do rostliny. Příjem prvků je ovlivněn enzymatickými procesy, koncentrací a formou, ve kterých se vyskytují, iontovou kompeticí a interakcí. Chelatační činidla jsou látky, které obsahují negativně nabité karboxylové skupiny nebo nukleofilní dusíkaté skupiny v takovém prostorovém uspořádání, že mohou vytvořit s kovovým iontem koordinační komplex, toto se nazývá chelatace. Chelatace mobilizuje kovy v půdě a také pomáhá transportovat kovový iont přes plazmatickou membránu jako komplex kov-chelát pomocí speciálních přenašečů. Syntéza chelátů je indukována nedostatkem kovu. Tento proces je významný hlavně u lipnicovitých rostlin. U jiných rostlin se na plazmatické membráně při nedostatku kovu aktivuje syntéza reduktázy, ta redukuje ionty kovů, které následně přenašeč dopraví přes plazmatickou membránu (McGrath et al. 2001).

Takto mobilizovaný iont kovu může vstoupit do rostlinné buňky apoplastickou nebo symplastickou cestou, záleží na typu kovu a druhu rostliny. V symplastické cestě dochází k přenosu iontu přes plasmatickou membránu pomocí specifických přenašečů. Dnes se již ví, že tyto přenašeče hrají důležitou roli v toleranci rostlin ke kovům (McGrath et al. 2001).

Dostane-li se kov do buňky, může být uložen v kořeni a detoxifikován nebo může být xylémem transportován do nadzemních částí rostliny. V buňce může být kov uložen ve vakuole nebo může být navázán na buněčnou stěnu. Přes tonoplast je kov transportován přenašečem buď jako volný iont nebo jako komplex kov – chelát. Transport xylémem probíhá opět za účasti chelátorů. Hlavním transportérem v xylému je nikotinamin. Transport z xylému do listových buněk je opět uskutečňován přes specifické přenašeče (McGrath et al. 2001; Hassan et al. 2008).

Chelátory mohou být různé organické kyseliny, včetně aminokyselin. Tyto látky jsou významné z hlediska odolnosti rostlin proti toxickému působení těžkých kovů. Největší množství těžkých kovů je akumulováno v kořenech, jen část je transportována do nadzemních částí (McGrath et al. 2001; Hassan et al. 2008).

Příjem zinku a kadmia rostlinami

Rostliny přijímají zinek převážně jako kationt Zn^{2+} a kadmium jako Cd^{2+}. Vysoké hladiny zinku a kadmia v půdě snižují příjem fosforu a železa (Yang et al. 2006; Zhang & Song 2006).

Zinek se v rostlinách vyskytuje jako volný ion, v komplexu s nízkomolekulárními sloučeninami, metaloproteiny a také zabudovaný do buněčných stěn (Kochian 1991). Tvoří komplexy s N, O a S-ligandy, čímž hraje katalytickou a strukturální úlohu v enzymatických reakcích. Podobně se chová kadmium s tím rozdílem, že průběh enzymatických reakcí zastavuje, neboť obsazuje ta vazebná místa, kam by se za normálních okolností navázal zinek (Vallee & Auld, 1990; Zhang & Song 2006).

Význam zinku pro rostliny

Zinek je, podobně jako v případě živočichů, nezbytný také pro správnou funkci životně důležitých biochemických procesů u rostlin (Peck & McDonald 2010; Hafeez et al. 2013).

Zn hraje velmi důležitou roli v metabolismu rostlin tím, že ovlivňuje činnost hydrogenázy a karboanhydrázy a podílí se na stabilizaci ribozomálních frakcí. Rostlinné enzymy aktivované Zn se podílejí na metabolismu sacharidů, udržování integrity buněčných membrán, syntéze proteinů, regulaci syntézy auxinu a tvorbě pylu. Regulace a udržování genové exprese potřebné pro zvládání environmentálních stresů v rostlinách jsou také procesy závislé na zinku (Peck & McDonald 2010; Hafeez et al. 2013).

Nedostatek zinku má za následek vznik abnormalit, jako je zastavení růstu, chloróza, menší listy, sterilitu klásku. Může také nepříznivě ovlivnit kvalitu sklizených produktů, náchylnost rostlin k poškození způsobenému vysokou intenzitou světla nebo vysokou teplotou a k infekci houbovým onemocněním. Zdá se, že zinek také ovlivňuje absorpci vody a její transport v rostlině. Zinek rovněž snižuje nepříznivé působení krátkých období nadměrného horka a stres způsobený vystavením rostlin vysoce zasoleným půdám. Protože je Zn nezbytný pro syntézu tryptofanu, který je prekurzorem kyseliny indol-3octové (IAA), hraje aktivní roli při produkci esenciálního růstového hormonu auxinu. Také je, podobně jako u živočichů, zinek nezbytný pro

udržení integrity buněčných membrán, tedy pro zachování struktury a orientace makromolekul a iontových transportních systémů, kdy je zásadní jeho interakce s fosfolipidy a thiolovými skupinami membránových proteinů (Peck & McDonald 2010; Hafeez et al. 2013).

Hyperakumulátory

Rostliny, které mají schopnost akumulovat těžké kovy ve svých tkáních, se nazývají hyperakumulátory. Genetický základ tolerance a hyperakumulace zinku byl zjištěn i u huseníčku Hallerova (*Arabidopsis halleri*), který je schopen akumulovat značné množství Zn a Cd v nadzemní části rostliny, a to v rozmezí 1 800 - 13 100 ppm (Bert et al. 2003).

Rovněž penízek modravý (*Thlaspi caerulescens*), drobná rostlinka z čeledi brukvovitých, hromadí ve svých listech těžké kovy. Dokáže akumulovat více jak 3 % zinku (v sušině) bez známek poškození. Penízek modravý je druh poměrně hojný na ruderálních stanovištích po celé Evropě. Často se vyskytuje právě v oblastech s vysokou koncentrací těžkých kovů v půdě, jako například na výsypkách dolů. Akumulace niklu, zinku a kadmia je u této rostliny formou obrany před bakterií *Pseudomonas syringae* pv. Maculicola (Fones et al. 2010).

Celkem je popsáno asi 15 druhů rostlinných hyperakumulátorů, z čehož 10 druhů patří do rodu Thlapsi (Munkhtsenseg et al. 2014).

Fytoremediace

Pod pojmem fytoremediace rozumíme proces, při němž jsou využívány rostliny k odstranění škodlivých látek z prostředí nebo k jejich transformaci (Cunningham et al. 1996). Přitom je využíváno přirozeně probíhajících procesů v rostlinách. Rostliny svým kořenovým systémem přijímají vodu a v ní rozpuštěné látky včetně např. těžkých kovů a různých chemikálií. Následně tyto látky buď transformují, nebo akumulují. V případě akumulace jsou rostliny sklizeny a např. spáleny v takovém prostředí, aby nedošlo k uvolnění kontaminantu zpět do prostředí. Tento proces vede k omezení množství toxicity, mobility nebo objemu kontaminantu. V dnešní době se k fytoremediaci využívají zejména rostliny, které

nazýváme hyperakumulátory. Ty jsou schopny akumulovat velké množství kovů (McGrath et al. 2001).

Při fytoremediaci je potřeba zajistit, aby nedocházelo k přeměnám na metabolity, které jsou toxičtější než samotný polutant. Technologie fytoremediace zahrnuje fytoextrakci, fytodegradaci, rhizofiltraci, fytovolatilizaci a fytostabilizaci (Bauddh et al. 2017; McGrath et al. 2001).

1. Fytoakumulace: kov je akumulován v nadzemní části rostliny, která je posléze sklizena.

Aby byla fytoextrakce účelná, je nutné, aby rostlina měla rychlý přírůstek biomasy a dokázala akumulovat velké množství kovu v nadzemních orgánech. Proto se při této metodě dobře uplatňují hyperakumulující rostliny. Pro zvýšení účinku fytoextrakce se většinou do půdy přidávají syntetické cheláty, např. kyselina ethylendiamintetraoctová (EDTA). Rostlina musí být také schopna tolerovat vysokou koncentraci daného kontaminantu, těžkého kovu.

2. Fytovolatilizace: způsob fytoremediace, kdy dojde k transpiraci těkavé formy kovu.

Takto může být odstraněn arsen, rtuť nebo selen, které existují právě i jako těkavé hydridy nebo methyl deriváty.

3. Fytostabilizace: zde je využíváno rostlin k imobilizaci vodní i půdní kontaminace kovu.

Kov nadále zůstává v půdě, ale jeho negativní vliv na prostředí je redukován. Účinek této metody je velmi závislý na chemických a fyzikálních vlastnostech půdy. Rostliny díky absorpci a adsorpci kořenů a precipitaci omezují migraci kovů v půdě a snižují jejich vymývání z půdy. Je zde využíváno redoxních reakcí nebo precipitace na nerozpustnou formu. Rostlinné kořeny mohou vylučovat látky, exudáty, které ovlivňují pH půdy a vyvázání kovu, většinou zvýšením pH.

4. Rhizofiltrace: dochází k odstranění kovu z vody a jeho akumulace v kořenech vodních rostlin.

Tato metoda je vhodná v případech velkých objemů vod s nízkou hladinou kontaminace. Je vhodná i pro odstranění radionuklidů. Vhodné rostliny pro tuto metodu, by měly mít rozsáhlý kořenový systém.

Huseníček Hallerův (*Arabidospis halleri*)

Huseníček Hallerův (*Arabidospis halleri*) je 20–60 cm vysoká výběžkatá bylina. Rostlina je porostlá jednoduchými nebo vícekrát rozeklanými trichomy. Lodyha je poléhavá, listy jsou vejčitého tvaru, řapíkaté, mívají zubaté, pilovité nebo vroubkované okraje. Oboupohlavné květy vytváří hroznové květenství. Okvětní lístky jsou bílé nebo světle fialové. Semena jsou podlouhlá, zploštělá a světle hnědá ve velikosti 0,5 – 0,7 mm. Kvete od května do srpna. Ve většině případů preferuje zastíněné lokality, lesní okraje, štěrkovité nebo travnaté svahy v nadmořské výšce 0–2600 m n. m. téměř po celé Evropě a východní Asii (O'Kane a Al – Shehbaz 1997). *Arabidopsis halleri* akumuluje více zinku než kadmia (Zhao et al. 2006).

1.4 Tasemnice a rizikové prvky

Parazitické organismy představují v dnešní době velmi zajímavý objekt výzkumu. Parazitologie jako samostatný vědní obor vznikla v roce 1877. Zvláštní způsob života i praktický význam parazitů vyvolává zájem o tyto organismy v řadě vědních oborů. Mnoho autorů se také snaží popsat různé faktory prostředí, které mají vliv na parazity (Olson et al. 2003; Volf & Horák 2007).

Existuje více formulací parazitismu, všechny ale v konečném výsledku dojdou ke shodnému závěru, který zní, že „parazitismus je vztah dvou organismů, kdy jeden žije na úkor toho druhého“. Parazit žije na úkor svého hostitele, avšak bezprostředně nepůsobí smrt. Je důležité si uvědomit, že pro parazity jsou biotopem samy živé organismy. Parazitismus je jedním z nejvýznamnějších faktorů ovlivňujících hostitelské populace volně žijících organismů. V přírodě je velmi rozšířen (Olson et al. 2003; Volf & Horák 2007).

Podle umístění můžeme parazity dělit na ektoparazity (klíště, komár) a endoparazity (tasemnice, škrkavka). Zatímco ektoparazité jsou bezprostředně ovlivňováni prostředím hostitele, v němž žije, endoparazité a jejich volně žijící stádia

jsou ovlivňováni nejen prostřednictvím svého hostitele, ale i prostředím případných mezihostitelů (Olson et al. 2003; Volf & Horák, 2007).

Tasemnice krysí (*Hymenolepis diminuta*)

Tasemnice krysí (*Hymenolepis diminuta*) dorůstá délky až 60 cm, výjimečně 90 cm. Živiny ze střeva hostitele absorbuje povrchem svého těla, takzvaným tegumentem. Její tělo je rozděleno na 3 části, scolex (hlavička), krk a zbytek těla tvoří články (strobillum). Na rozdíl od některých jiných tasemnic nemá na hlavičce (scolexu) háčky, kterými by zraňovala tkáň svého hostitele. Každý jedinec tasemnice obsahuje samičí i samčí reprodukční orgány. Jedna dospělá tasemnice *Hymenolepis diminuta* je schopna ve střevě svého hostitele produkovat až 250 000 vajíček denně (Sulima-Celińska et al. 2022; Olson et al. 2003; Volf & Horák 2007).

Tato tasemnice se vyskytuje u hostitelů, hlavně hlodavců, někdy však také u psů a lidí, žijících v mírném zeměpisném pásmu celého světa. Mezihostiteli jsou brouci, potemníci (*Tribolium molitor*, *Tenebrio opacus*), kteří se nakazí vajíčky tasemnice, která jsou jimi pozřeny spolu s trusem potkanů. K nakažení konečného hostitele i mezihostitele dochází nejčastěji v obilí, kde oba živočichové žijí. Vajíčka tasemnic se v mezihostiteli vyvinou do stádia cysticerkoidů. Potkan se poté nakazí pozřením infikovaného brouka (Arai 1980).

Infekce tasemnicí *Hymenolepis diminuta* člověkem je doprovázena bolestmi hlavy, emocionální podrážděností, enteritidou a také může způsobit anorexii. Člověk se může nakazit stravou kontaminovanou infikovanými brouky (Sulima-Celińska et al. 2022).

Vliv infekce tasemnice krysí (*Hymenolepis diminuta*) na transport iontů ve střevech potkana obecného (*Rattus norvegicus*)

Hymenolepis diminuta je parazit tenkého střeva hlavně u hlodavců (především myší a potkanů), ale náhodně může být i u lidí. Nemá žádné háčky, které by mohly mechanicky poškodit hostitelské tkáně a jako takový je ve střevě neinvazivní. Nicméně metabolity produkované *H. diminuta* narušují činnost trávicího traktu, zvyšují sekreci slin, inhibují sekreci v žaludku a zvyšují aktivitu trypsinu v chymu v duodenu. U nakažených potkanů také byly pozorovány patomorfologické

změny v tenkém střevě, akutní zánětlivá reakce a značné poškození svaloviny. Chronická fáze infekce *H. diminuta* vede k vymizení střevních klků, což má za následek narušení závěrečných fází procesu trávení a vede k malabsorpci (porucha vstřebávání živin). Bylo zjištěno, že u potkanů infikovaných *H. diminuta*, je celé tenké střevo ovlivněno kvůli velikosti tasemnice (Sulima-Celińska et al. 2022; Kosik – Bogacka et al. 2010).

Taktéž bylo prokázáno, že tasemnice krysí (*Hymenolepis diminuta*) inhibuje transport iontů sodíku a chloridů v epitelu tlustého střeva potkana a má vliv na snížení mechanické citlivosti střevního epitelu (Kosik – Bogacka et al. 2010).

Infekce tasemnicí ovlivňuje krevní obraz. Bylo zjištěno podstatně nižší množství červených krvinek (erytrocytů) a nižší koncentrace hemoglobinu oproti neinfikovaným potkanům. Počet červených krvinek a krevních destiček koreluje s délkou infekce, a to tím způsobem, že jejich množství klesá, avšak objem erytrocytů s délkou infekce roste. Stejně tak množství bílých krvinek (leukocytů), hlavně eozinofilů a basofilů u infikovaných potkanů je podstatně nižší než u potkanů bez parazita. Výsledky naznačují, že infekce tasemnicí *H. diminuta* je spojena s aktivací zánětlivých mediátorů a stimulací nervových vláken, což výrazně ovlivňuje funkci iontových kanálů v epitelu tlustého střeva v hostiteli (Starke et al. 2001; Kosik – Bogacka et al. 2010).

Infekce trávicího traktu parazity tedy vyvolává imunologickou odpověď a patofyziologické změny v hostitelích. Vzhledem k úzkému vztahu mezi epitelovou buňkou v trávicím traktu a místním imunitním systémem, mohou místní imunitní reakce změnit transport iontů v epitelu, což způsobuje zvýšenou sekreci, snížení vstřebáváni iontů nebo obojí. Přítomnost tasemnice má tedy vliv na přenos iontů těžkých kovů do krevního řečiště (Kosik – Bogacka et al. 2010).

Tasemnice mají značnou schopnost akumulovat těžké kovy v koncentracích přesahující koncentrace těchto kovů v tkáních jejich hostitelů. U tasemnic chybí trávicí soustava a živiny přijímají celým, metabolicky aktivním povrchem těla (tegumentem). O jednotlivé prvky včetně těžkých kovů soupeří se střevní stěnou hostitele. Tasemnice jsou hojným parazitem u suchozemských zvířat a mohou tak být užitečné při biomonitoringu. Například tasemnice rodu Hymenolepis jsou skupinou parazitů nalézaných ve střevech hlodavců dokonce i v městských ekosystémech (Sures 2003).

2 Cíl a hypotéza

Cíl práce

Ověřit, zda je tasemnice schopna ovlivnit množství zinku a kadmia

- v tkáních,
- v moči,
- ve výkalech,

hostitele, potkana.

Hypotéza práce

Tasemnice ovlivní množství zinku a kadmia ve výkalech, moči a tkáních hostitele.

3 Publikované práce

3.1 Publikace uvedené v individuálním studijním plánu

1) Sloup V, Jankovská I, Száková J, Magdálek J, Sloup S, Langrová I. 2018. Effects of tapeworm infection on absorption and excretion of zinc and cadmium by experimental rats. Environ. Sci. Pollut. Res., **25**:35464–35470. https://doi.org/10.1007/s11356-018-3397-9 **(Q2 dle AIS)**

2) Sloup V, Jankovská I, Száková J, Karešová V, Lanková S, Sloup S, Langrová I. 2021a. Excretion of dietary zinc in mammals (rats) fed overdoses of zinc lactate and infected with tapeworms. Helminthologia, **58**:339–345. https://doi.org/10.2478/helm-2021-0038 **(Q4 dle AIS)**

3) Sloup V, Jankovská I, Štolcová M, Magdálek J, Karešová V, Lanková S, Langrová I. 2021b. Effects of excessive dietary zinc or zinc/cadmium and tapeworm infection on the biochemical parameters in rats. J. Anim. Physiol. Anim. Nutr., **105**:989–995. https://doi.org/10.1111/jpn.13524 **(Q2 dle AIS)**

4) Jankovská I, Karešová V, Michlová T, Kunc P, Knížková I, Ševčík R, Sloup V, Langrová I. 2023. Significance of Intestinal Helminth Infection and Animal Sex for Mercury Concentrations in Two Rodent Species. Journal of Wildlife Diseases **59**:504-508. https://doi.org/10.7589/JWD-D-22-00129 **(Q2 dle AIS)**

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RESEARCH ARTICLE

Effects of tapeworm infection on absorption and excretion of zinc and cadmium by experimental rats

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Abstract
The main objective of this study was to determine how rat tapeworms affect the excretion of zinc and cadmium through rat feces. Male rats (*Rattus norvegicus* var. *alba*) were divided into four groups, and the experiment was conducted over a 6-week period. The control groups (00; 0T) were provided with a standard ST-1 rodent mixture and received 10.5 mg of Zn/week. Groups P0 and PT were fed a mixture supplemented with the hyperaccumulating plant *Arabidopsis halleri* at a dosage of 123 mg Zn/week and 2.46 mg Cd/week. Groups 0T and PT were infected with the rat tapeworm (*Hymenolepis diminuta*). Fecal samples were collected 24 h post exposure. Zinc and cadmium concentrations in rat feces were analyzed using inductively coupled plasma optical emission spectrometry. Tapeworm presence decreased the amount of metals excreted through the feces of the host throughout the entire experiment, with the exception of 1 week (control group). No statistically significant differences between zinc excretion rates in the control groups (00 and 0T) were detected at any time throughout the experiment. A statistically significant difference between zinc excretion rates ($p < 0.05$) in the exposed groups (P0 and PT) was detected in 2 of the 6 monitored weeks. Group PT excreted significantly less cadmium ($p < 0.01$) than group P0 did in three of the 6 weeks. Overall, our results indicate that tapeworms are able to influence the excretion of metals by their host. Tapeworms accumulate metals from intestinal contents. It is not clear whether tapeworms carry out this process before the host tissues absorb the metals from the intestines or the tapeworms accumulate metals excreted from the body of the host back to the intestines. Most likely, it is a combination of both phenomena.

Keywords Zinc · Cadmium · Tapeworm · Excretion · Feces · Rat · Hyperaccumulators

Introduction

Zinc (Zn) and cadmium (Cd) are among the heavy metals that constitute an ill-defined group of inorganic chemical hazards (Wuana and Okieimen 2011).

Zinc can be toxic in high doses. In cases where nutritional supplements enriched with Zn are taken in high doses, the metabolism of other metals may be disturbed. When Zn is taken in extremely high doses, it is absorbed in the intestines at the expense of other metal ions, and the levels of Cu, Fe, Co, or Cr decrease. If this process continues unchanged, symptoms of anemia can arise (Ferguson et al. 1995; Brody 1998).

Zinc is one of the most important nutrients for animal health. Numerous proteins, crucial enzymes, and transcription factors bind to Zn and are thought to be dependent on Zn for their functions. Zn is involved in many biochemical processes that support life (Brown et al. 2001; Cao et al. 2016). Zinc, the most abundant intracellular element, is found in all body tissues, with ~ 85% of whole body zinc concentrations found in the muscle and bone, 11% in the skin and liver, and the remaining 2 to 3% in all other tissues (Jackson 1989).

Unlike zinc, cadmium is purely toxic to an animal organism. The retention and toxicity of Cd depends on various factors, such as daily intake, forms of Cd in food, its interactions with essential elements, and the nutritional status of the population (Chmielnicka and Cherian 1986). At low levels of exposure, cadmium is accumulated in the kidneys. Renal

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dysfunction may develop if critical levels are reached in renal tissue. Other adverse health effects seen following exposure to cadmium include bone disorders, e.g., osteoporosis and its more severe forms such as osteomalacia found in itai-itai (cadmium poisoning) patients (Jin et al. 2002; Zheng et al. 2014). Oral ingestion of toxic metals such as cadmium perturbs the metabolism of essential elements, especially zinc, copper, iron, and selenium (Abdulla and Chmielnicka 1989). Cadmium has been classified as a human carcinogen and exerts toxic effects on the central nervous system. In addition, these effests are mutagenic, teratogenic, and embryotoxic (Winiarska-Mieczan and Kwiecień 2016).

Both metals can be accumulated by plants known as Hyperaccumulators (Bayçu et al. 2017). Hyperaccumulators possess high amounts of heavy metals, including zinc and cadmium, in their tissues (Hesami et al. 2018). These metals can then access the food chain through these plants and from them, metals can get into the food chain. *Arabidopsis halleri* is a pseudo-metallophyte species, which means that it occurs on both contaminated and noncontaminated sites. As a Zn hyperaccumulator, *A. halleri* can accumulate Zn in extremely high amounts in its aerial parts. In addition, this species has a widespread distribution throughout Europe. *A. halleri* was found to accumulate not only Zn but also Cd in its shoot biomass. *A. halleri* is considered an appropriate model plant for studying metal tolerance and hyperaccumulation (Sarret et al. 2009). Moreover, this species can also be used in phytoremediation and heavy metal monitoring in the environment (Zhenli et al. 2005).

Animals receive nutrients through their digestive tracts. These nutrients then come into contact with gastrointestinal parasites, including tapeworms. Tapeworms receive nutrients through the tegument, a metabolically active body surface, in addition metal elements (Chowdhury and Singh 1989). Tapeworms are able to accumulate high amounts of metals, and this leads to a decrease in the amount of metals in a host; this decrease is most evident in the organs, as many studies indicate (Jankovská et al. 2010a, b, 2016, 2018). Currently, there are very few studies dealing with the ability of tapeworms to decrease heavy metal levels in a host. It is precisely this issue that we decided to focus on in our study.

Material and methodology

Maintenance of experimental animals

Twenty-five male Wistar rats (*Rattus norvegicus* var. *alba*) were used in this study (Velaz, Prague, Czech Republic), and they each had an initial weight of 150 g. Each animal was placed in its own metabolic cage. The room housing the cages was equipped with air conditioning. A constant temperature (22–24 °C), humidity level (approximately 70%), and day/night cycle (08:00 am–08:00 pm) were maintained. During the acclimatization period, the animals were fed a standard mixture for rats (ST-1 by Velaz, Prague, Czech Republic) (Table 1) and given ad libitum access to water.

During the acclimatization period, a number of rats were infected with a rat tapeworm (*Hymenolepis diminuta*). The beetle *Tribolium confusum* served as the intermediate host. Cysticercoid development in the beetles took place over a 12-day period in an incubator at a temperature of 29 °C. Subsequently, the cysticercoids were extracted from the beetles and fed to the rats (approximately three cysticercoids per rat). The infection success rate was evaluated by a coprological examination carried out 5 weeks post infection. The existence of eggs in the rat feces confirmed the presence of tapeworm infection.

Experimental design

After 5 weeks of acclimatization, the rats were divided into four groups based on the tapeworm infection success rate. The control groups (00; 0T) were provided with a standard ST-1 rodent mixture and received 10.5 mg of Zn/week. Groups P0 and PT were fed a mixture supplemented with the hyperaccumulating plant *A. halleri* containing metals at a dosage of 123 mg Zn/week and 2.46 mg Cd/week. Groups 0T and PT were infected with the rat tapeworm (*H. diminuta*). The rats were each given 25 g of food Monday through Thursday and 50 g on Fridays. Groups 00 and 0T were given ST-1 standard feed for rodents (Table 1), and each 25-g feed dose contained 1.75 mg of Zn. Group 0T was infected with *H. diminuta*. Groups P0 and PT were fed ST-1 with an admixture of *A. halleri* (Příbram, Czech Republic). Twenty-five grams of the mixture contained 20.5 mg of zinc and 0.41 mg of cadmium (Table 2). Group PT was infected with *H. diminuta*. Feed consumption was recorded daily. The entire experiment lasted 6 weeks.

Analytical procedures

All fecal samples were placed into plastic boxes and stored at − 20 °C until chemical analysis. Frozen samples were freeze-dried (LYOVAC GT 2: LEYBOLD-HERAEUS, GmbH, Germany) and ground into a fine powder. The aliquots of the samples were decomposed by microwave-assisted pressurized wet digestion in a mixture of hydrogen peroxide (35%) and nitric acid (65%).

Concentrations of Cd in the digests were determind by atomic absorption spectrometry with electrothermal atomization using a Varian AA 280Z spectrometer (Varian, Australia) equipped with graphite tube atomizer GTA 120 and PSD 120 programmable sample dispenser. Concentrations of Zn in the digests were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA) equipped with a two-channel

Table 1 Composition of ST-1 (commercially available from Velaz Ltd. CR)

Moisture (%)	12.5
Nitrogen compounds (%)	24
Fiber (%)	4.4
Lipids (%)	3.4
Ash (%)	6.8
Lysin (mg/kg)	14,000
Methionine (mg/kg)	4800
Ca (mg/kg)	11,000
P (mg/kg)	7200
Na (mg/kg)	1800
Cu (mg/kg)	20
Zn (mg/kg)	70
Se (mg/kg)	0.38

peristaltic pump, a Struman–Masters spray chamber, and a V-groove pneumatic nebulizer made of inert material.

For quality assurance of the analytical system, simultaneous analysis of a certified reference material BCR 185R bovine liver (4% of all the samples) was provided. Analytical data obtained for all determined elements were within in the confidence interval given by the producer. The background of the trace element laboratory was monitored by analysis of 15% blanks prepared under the same conditions, but without samples, and experimental data were corrected by mean concentration of the elements in blanks, and compared with detection limits (mean ± 3 SD of blanks) which were 0.08 ng mL-1 for Cd and 7.5 ng mL-1 for Zn.

Statistical analysis

We used a nonparametric Mann–Whitney U test for evaluation of the proposed hypothesis. There was a P value = 0.05 for our hypothesis about the significant statistical influence of zinc and cadmium in rat food. Statistics software Statistica 10 (Statsoft, USA) was used for all computations and statistical analysis.

Results

Over the course of the experiment, 150 fecal samples were taken and analyzed. Summary results are presented in Table 3.

It is clear that fecal excretion serves as the main vehicle for the elimination of both metals. Group 00 and group P0 had the lowest and highest mean zinc excretion values, respectively. Cadmium was not evaluated in the control groups (00 and 0T). Zinc and cadmium elimination through the feces ranged from 61 to 78.1% (Table 3). Group 0T excreted 1% more zinc than group 00 did. These results are evident only in the case of a summary evaluation over the entire duration of the experiment and when results are calculated using a median. From the evaluation of individual weeks of the case where the mean is used, it is evident that only in the second week the rats from group 00 excreted more zinc than rats from 0T group. In the other 5 weeks, the influence of tapeworm appeared; however, statistically significant difference was not found in any case (Fig. 1).

The influence of tapeworms was evident in the exposed groups (P0 and PT) for zinc and cadmium. A statistically significant difference between these two groups was found in zinc excretion rates in 2 of the 6 weeks. The PT group rats excreted statistically less ($P < 0.05$) zinc compared to that of the P0 group, specifically in the first and third weeks (Fig. 2).

Tapeworms seemed to exert the most influence on the elimination of cadmium through feces. The tapeworm-infected group (PT) excreted significantly less cadmium than the uninfected group did (P0). A statistically significant difference ($P < 0.01$) was observed in 3 of the 6 weeks. In the remaining 3 weeks, the infected rats (PT) excreted less cadmium; however, the difference was not statistically significant (Fig. 3).

Discussion

In our research, we focused on levels of zinc and cadmium excreted in the feces after consumption of a plant hyperaccumulator. We also observed how tapeworms can influence the amount of metals excreted by their host through the feces.

According to Klaassen and Kotsonis (1977), elimination of cadmium via feces is more effective than via urine. Regulation of zinc excretion through the gastrointestinal tract is the most significant mechanism in maintaining zinc homeostasis (Lee et al. 1993). Unabsorbed zinc appears in feces through food comsuption, endogenous zinc excreted from the gallblader

Table 2 Zinc and cadmium contents in feed

Experimental group	Number of animals	Infection tapeworm	Zinc dose (mg/25 g food)	Zinc dose/week (mg)	Cadmium dose (mg/25 g food)	Cadmium dose/week (mg)
00	6	–	1.75	10.5		
0T	7	+	1.75	10.5		
P0	6	–	20.5	123	0.41	2.46
PT	6	+	20.5	123	0.41	2.46

Table 3 Zinc and cadmium dosages and levels of Zn and Cd excreted in feces (mg) over a 6-week period

Experimental group	Zinc intake (mg/day)	Fecal excretion zinc (mg/day)	Cadmium intake (mg/day)	Fecal excretion cadmium (mg/day)	Fecal excretion zinc (%)	Fecal excretion cadmium (%)
00	1.75 ± 0.17	1.23 ± 0.19			69.7	
0T	1.67 ± 0.18	1.18 ± 0.19			70.7	
P0	14.80 ± 4.24	11.56 ± 3.75	0.30 ± 0.09	0.21 ± 0.08	78.1	70.0
PT	20.50 ± 1.64	15.49 ± 2.25	0.41 ± 0.03	0.25 ± 0.04	75.6	61.0

Values expressed as the median ± standard deviation in the group

and pancreas into the intestine, zinc excreted through gastroduodenal secretions, and zinc excreted from mucosal cells (Erdman et al. 2012). Therefore, tapeworms come into contact with metals received through food as well as with metals excreted from the body.

Figures 1 and 2 show a reduction in excreted zinc during the first 2 weeks, especially in the uninfected groups (00; P0). We assume that less zinc was absorbed during these first 2 weeks than in the following weeks. Research carried out by King et al. (2000) confirms different quantities of excreted zinc depending on the length of exposure. It seems that the amount of excreted zinc has an upward trend over time. It is possible, however, that our rats were excreting zinc that had been accumulated in the first 2 weeks. Klaassen and Kotsonis (1977) found that 85% of cadmium was excreted in feces within 2 days. The same thing can be assumed about zinc. Zinc administered during the first 2 weeks of our experiment most likely did not affect levels of zinc excreted in the remaining weeks.

In another study, the authors found a connection between ingested doses of zinc and absorbed zinc (feces Zn excretion). King et al. 2000 demonstrated that the efficiency of zinc absorption decreased from nearly 100% with a zinc-free diet to 55% with 0.5 mg of added zinc daily. Endogenous fecal zinc excretion varied 30-fold. In our study, rats from the control groups (00; 0T) received a maximum of 1.75 mg Zn/day and excreted from 67 to 79% of zinc (Fig. 1) through the feces (rate of absorption from 21 to 33%). Rats from the exposed groups (P0; PT) received a maximum of 20.5 mg Zn/day and excreted from 69 to 90% of zinc (Fig. 2) through the feces (rate of absorption, 10–31%). This confirms the ability of an organism to manage zinc effectively and to regulate its intake and excretion through the gastrointestinal tract.

Cikrt and Tichý (1974) similarly confirm the difference in levels of excreted cadmium at various doses. Over a 24-h period, 0.83% of Cd was excreted from a dose of 67 μg, 1.18% of Cd was excreted from a dose of 90 μg, and 5.68% of Cd was excreted from a dose of 120 μg. In our study, rats received a maximum of 0.41 mg of Cd. Cadmium excretion rates ranged from 71 to 82% and 58 to 71% for group P0 and group PT, respectively. Decker et al. (1957) gave 2 mg of Cd to rats orally, an amount approximately five times as high as that used in our study. They found that more than 90% of the given dose was excreted through feces, with only a small percentage absorbed. This confirms the relationship between the size of the dose, the absorption rate, and the amount of Cd excreted in feces. According to Godt et al. (2006), approximately 5% of ingested cadmium is taken up through the gastrointestinal tract, depending on the exact dose and nutritional composition. In our study, the rate of absorption reached 42%.

Čadková et al. (2013) described the importance of the metal source (organic or inorganic) in terms of absorption and excretion. In their experiment, lead oral exposure was

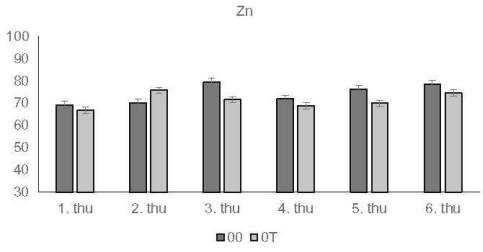

Fig. 1 A comparison of Zn excretion rates (%) in the feces of uninfected control rats (00) and those of control rats (0 T) infected with tapeworms (*Hymenolepis diminuta*). thu, Thursday (samples were taken every Thursday)

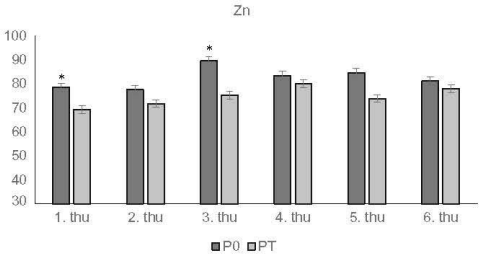

Fig. 2 A comparison between Zn excretion rates (%) in the feces of rats from group P0 (uninfected and fed *Arabidopsis halleri*) and those of rats from group PT (tapeworm-infected and fed *Arabidopsis halleri*).*Statistically significant difference between groups P0 a PT, $p < 0.05$; thu, Thursday (samples were taken every Thursday)

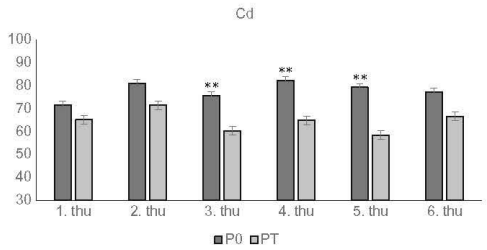

Fig. 3 A comparison between Cd excretion rates (%) in the feces of rats from group P0 (uninfected and fed *Arabidopsis halleri*) and those of rats from group PT (tapeworm-infected and fed *Arabidopsis halleri*). **Statistically significant difference between groups P0 a PT, $p < 0.01$; thu, Thursday (samples were taken every Thursday)

administered in two different forms—lead acetate and phytobound Pb (*Pistia stratiotes*). The results show that up to 53% of ingested Pb was rapidly eliminated from the exposed rats via feces within 24 h after exposure. Considerable differences were revealed concerning total excretion levels; lead acetate was excreted in greater amounts than those of phytobound Pb. Lead was not evaluated in our study; however, cadmium and zinc behave similarly in an animal body (Petering 1979). We can assume that zinc and cadmium are bound in *A. halleri* in a similar form in which lead is bound in *P. stratiotes*. Isaure et al. (2015) state that zinc is bound in a plant hyperaccumulator primarily as a Zn-organic acid complex. According to Huguet et al. 2012, cadmium binds primarly to organic acids in plants that accumulate cadmium.

According to Roohani et al. (2013), 70 to 80% of consumed zinc is excreted through feces. In our study, 67 to 79% and 71 to 91% of Zn were excreted through feces within 24 h in groups 00 and 0T and groups P0 and PT, respectively. However, groups P0 and PT received 12 times as much zinc as groups 00 and 0T did. That may be related to better absorption of zinc bound in the plant. The exposed groups (P0; PT), however, excreted more zinc than the control groups (00; 0T) did, although this may be related to the relationship between the given dose and the amount of excreted metal.

However, the claim that metals bound in plants are easily absorbable seems to conflict with the claim that phytic acid is considered an antinutritional substance, which reduces the use of certain substances, including zinc, in animals and people (House et al. 1982; Milne et al. 1984).

Cadmium and zinc are very similar elements from a chemical and physical standpoint (Das et al. 1997; Chaney 2010; Tang et al. 2014), and therefore influence each other. Both metals bind to the same proteins once they are absorbed in the digestive tract—albumin in the blood stream, metallothionein (MT), and other proteins in the tissues (Brzóska and Moniuszko-Jakoniuk 2001). A high intake of Zn and Cd stimulates synthesis of cysteine-rich MT in small intestinal mucosa (Kägi 1991). Binding to MT reduces the toxic effects of these two metals (Kelly et al. 1996). According to Funk et al. 1987, the synthesis of MT in the form of Cd5Zn2MT leads to a transfer of this form of MT from the blood plasma to the liver and kidneys, and to a subsequent excretion of metals from the liver to the intestines. We can consider the possibility that both metals were excreted into the intestines in a different form than they were in the control groups (00; 0T), because groups P0 and PT were exposed to both metals at the same time. In addition, metals in this form are more easily absorbed by tapeworms; this could explain the greater difference in zinc excretion between groups P0 and PT than between groups 00 and 0T. This presumption is supported by findings which indicate that some parasites can better accumulate certain substances. For instance, the tapeworm *Diphyllobothrium latum* absorbs large amounts of vitamin B_{12} in the gut of a host, and as a result, a large quantity of cobalt is accumulated by the parasite (Brand von 1973). This tapeworm can absorb a Zn-organic acid complex from a plant hyperaccumulator (Isaure et al. 2015) more easily than it can in zinc added to basic feed in the form of inorganic zinc salt.

Studies focusing on zinc and cadmium excretion through feces of animals infected by tapeworm are not available. However, many published studies have centered on the ability of tapeworms to accumulate heavy metals and thereby reduce their concentrations in tissues of their host. Tissues play an essential role in zinc metabolism. They are crucial for the intake and excretion of this metal. Tissue and cellular redistribution of zinc may contribute to the maintenance of zinc homeostasis (Lee et al. 1993).

According to Brand von (1973), 19.2% of the common tapeworm body is composed of inorganic substances. Our results indicate that the tapeworm has more of an influence on cadmium excretion than it does on zinc excretion in its host. This can be caused by the tapeworm's greater affinity for cadmium than for zinc. Zinc and Cd are chemically similar, and Cd can substitute Zn relatively easily in biological systems (Elinder and Piscator 1978; Tang et al. 2014). However, the exposed groups (P0; PT) received significantly more zinc than cadmium. The relatively low intake of cadmium by the tapeworms was enough to create a significant difference between levels from the infected group and those of the uninfected group.

According to Brožová et al. (2015), Cd concentrations in the small intestinal tissue of red foxes (*Vulpes vulpes*) infected by the tapeworm *Echinococcus multilocularis* were lower than those in uninfected red foxes. Zinc concentrations in the small intestinal tissue of infected red foxes, however, were higher than those of their uninfected counterparts.

Jankovská et al. (2010c) conducted experimental studies on the cadmium accumulation in the cestode *Moniezia expansa* (Cestoda: Anoplocephalidae) and its final host (*Ovis aries*).

They found higher concentrations of Cd in tapeworms than in all of the monitored sheep organs, with exception of the liver. The abovementioned authors also found that sheep infected with this tapeworm had higher concentrations of Cd in their tissues than the uninfected sheep did. In our study, the levels of excreted cadmium were significantly lower in rats infected by tapeworms throughout the entire 6-week duration. This means that either the tapeworms accumulated high levels of cadmium or, in accordance with the results of Jankovská et al. (2010c), the presence of the tapeworm caused the host tissues to store more cadmium than normal.

As we can see in Figs. 1 and 2, throughout the entire 6 weeks, the infected rats (group PT and 0T) excreted lower levels of zinc than the uninfected rats (groupS P0 and 00) did; the only exception to this result was in the second week, when the infected rats excreted more zinc. These findings are in accordance with the results of Jankovská et al. (2010a); in that study, higher levels of zinc were observed in parasites than in the host tissues. It is therefore possible that the tapeworms affected fecal zinc and cadmium levels by accumulation.

Conclusions

Many studies dealing with influence of parasites on the accumulation of risk elements (metals) in a host have been published. However, our study is one of few to observe how tapeworms influence the excretion of risk elements (Cd and Zn) through host feces. Our results indicate that tapeworms are able to influence the excretion of metals by their host. Tapeworms accumulate metals from intestinal contents. It is not clear whether tapeworms carry out this process before the host tissues absorb the metals from the intestines or the tapeworms accumulate metals excreted from the body of the host back to the intestines. Most likely, it is a combination of both phenomena. In any case, this is a significant topic for further research.

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Compliance with ethical standards

All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic Act No 246/1992 Coll. on the Protection of Animals against Cruelty.

Conflict of interest The authors declare that they have no conflict of interest.

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Excretion of dietary zinc in mammals (rats) fed overdoses of zinc lactate and infected with tapeworms

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Summary

Tapeworms parasitize at sites that are important for the management of micronutrients, including zinc. Therefore, it has been hypothesized that tapeworms will significantly affect the excretion of zinc in the feces of a host. The aim of this work was to evaluate the effects that tapeworms have on the excretion of zinc in the feces of the host. Rats were divided into 4 groups: groups 0T and MT (infected with *Hymenolepis diminuta* (Rudolphi, 1819)) and groups 00 and M0 (uninfected). The experimental groups (M0 and MT) were fed a standard rodent compound feed (ST-1) with added zinc lactate; the daily zinc intake was 20.5 mg. The control groups (00 and 0T) were fed only ST-1 with 1.75 mg of added Zn per day. For six weeks, the amount of consumed feed was recorded and fecal samples were taken. The samples were then analyzed by optical emission spectrometry (ICP-OES), and levels of excreted zinc were subsequently calculated as a percentage. The most significant difference in zinc excretion levels between the experimental groups was observed in the third week, when rats infected with tapeworms (MT) excreted substantially lower levels of zinc than did uninfected rats (M0). This difference amounted to 28.36 % ($p < 0.01$). In the control groups, tapeworms affected the excretion of zinc in the feces to a lesser extent, and the most substantial difference in zinc levels was seen in the fifth week (8.46 %). However, there was no significant difference in zinc excretion levels between the control groups during any of the monitored weeks. Tapeworms in the host affect levels of zinc excreted in the feces. However, this is dependent on the amount or form of zinc ingested.
Keywords: zinc; excretion; fecal; tapeworm; rat; supplement

Introduction

Zinc is present in all biological systems and, owing to its versatile physicochemical properties, performs various functions in the body. Zinc is involved in the metabolism of insulin (Khoobbakht *et al.*, 2020), carbohydrates, lipids and proteins. Zinc forms part of the digestive enzyme, and it is an important antioxidant which removes free radicals and protects cell membranes against lipid peroxidation. It significantly contributes to the growth and development of the individual as well as the proper functioning of the immune and reproductive systems (Williams, 2012; Gonçalves-Neto *et al.*, 2011). Zinc is released from food in the form of free ions. This occurs primarily in the distal duodenum and proximal jejunum. Approximately 60 % of absorption occurs in the duodenum, 30 % in the ileum, 7 % in the jejunum, and roughly 3 % in the appendix and colon (Hotz *et al.*, 2004). Zinc is absorbed by enterocytes through the basolateral membrane and then passes into the portal circulation. The portal system directs the zinc to the liver, from where it

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Table 1. Zinc contents in feed.

Experimental group	Number of animals	Infection tapeworm	Zinc dose (mg/25 g food)	Zinc dose/week (mg)
00	6	-	1.75	10.5
0T	6	+	1.75	10.5
M0	6	-	20.5	123
MT	6	+	20.5	123

is released into the bloodstream, eventually reaching other tissues (Hotz *et al.*, 2004). However, if zinc is ingested in high amounts, it is absorbed at the expense of other trace elements, and this may be expressed as a reduction in their uptake. This leads to a disruption of the metabolic processes that are dependent on these elements (Ferguson *et al.*, 1995).

Zinc excretion takes place mainly through feces, which contains unabsorbed zinc from food consumption, zinc present in intestinal epithelial cells, as well as endogenous zinc, which is excreted into the intestine by the gallbladder, pancreas, and gastroduodenal secretions (Erdman *et al.*, 2012). Approximately 70 to 80 % of ingested zinc is excreted in this way (Davies & Nightingale, 1975).

There is also evidence that zinc exerts a negative effect on ecosystems, basic soil functions and species diversity among microorganisms. This can, in turn, negatively affect the conversion of nutrients in the soil. In order to ensure protection of the environment and public health, a number of steps have been taken to reduce heavy metal emissions in recent years. However, such steps have not been applied to zinc. Zinc continues to be a common additive in compound feed. For example, zinc is given to newly weaned piglets in order to reduce the incidence of diarrhea (Jensen-Waern *et al.*, 1998) and improve both growth parameters and daily weight gain. However, such use of zinc leads to its accumulation in the environment and also to the development of bacterial resistance in LA-MRSA (livestock associated methicillin resistant *Staphylococcus aureus* (Rosenbach, 1884)). In Denmark, an increase in soil zinc concentrations of more than 45 % was recorded between 1998 and 2014. Based on those results, European agencies (EMA, EFSA) are reviewing the current permitted levels of zinc in compound feed (e.g. Directive 2016/1095) (Jensen *et al.*, 2018).

Zn in animals is taken up orally and subsequently absorbed and excreted mainly by the digestive tract. Since gastrointestinal helminths (including tapeworms) are present in the digestive tract, they can influence zinc metabolism significantly. According to many authors, tapeworms of the genus Hymenolepis can accumulate significant amounts of metal ions taken up by the host (Sures *et al.*, 2002; Jankovská *et al.*, 2018).

Our study, therefore, focused on comparing the levels of zinc ingested and excreted by rats infected with tapeworms to those ingested and secreted by uninfected rats. In addition to being given the standard dose of zinc, some rats received a zinc lactate additive. Thus, it has been hypothesized that tapeworms will significantly affect the excretion of zinc in the feces of a host.

Material and Methodology

Maintenance of experimental animals

Twenty-four male Wistar rats (*Rattus norvegicus domestica*) were used in the study. The average initial weight of each rat was 150 g. Each animal was placed in its own metabolic cage for the duration of the experiment. The room housing the cages was equipped with air-conditioning. A constant temperature (22 – 24 °C) and relative humidity level (approx. 70 %) were maintained at a constant day/night cycle (08:00 am – 08:00 pm). The animals had access to water *ad libitum* during the experiment. They were fed a standard mixture for rodents (ST-1 by Velaz, Prague, Czech Republic, see Table 2).

Rat tapeworm infection

During acclimatization, some rats were infected with the tapeworm *H. diminuta*. Tapeworm intermediate hosts were used for infection, namely beetles of the species *Tribolium confusum* (Jacquelin du Val, 1868). The beetles were infected by consuming the tapeworm eggs. Tapeworm larvae (cysticercoids) developed in beetles in an incubator over 12 days at 29 °C. After this time, the cysticercoides were extracted from the beetles and administered to rats orally

Table 2. Composition of ST-1 (commercially available from Velaz Ltd. CR).

Moisture (%)	12.5
Nitrogen compounds (%)	24
Fiber (%)	4.4
Lipids (%)	3.4
Ash (%)	6.8
Lysin (mg/kg)	14 000
Methionine (mg/kg)	4 800
Ca (mg/kg)	11 000
P (mg/kg)	7 200
Na (mg/kg)	1 800
Cu (mg/kg)	20
Zn (mg/kg)	70
Se (mg/kg)	0.38

using pipettes filled with a sucrose solution (three larvae per rat). Success of infection was determined by a coprological examination carried 5 weeks after infection.

Experimental Design

After acclimatization, the rats were divided into four groups (see Table 1). Each rat received 25 g of food per day for every day of the experiment (6 weeks), with the exception of Fridays, when they were given 50 g and then received another dose of food, again on Monday, again 25 g. Group 00 (no tapeworm infection) rats were fed a standard rodent compound feed (ST-1) during the experiment. The daily feed ration (25 g) contained 1.75 mg of Zn. Group 0T rats were fed the same mixture as those of group 00, but they were infected with *H. diminuta*. Group M0 (no tapeworm infection) rats were fed ST-1 with zinc lactate (p.a. grade, Lachema, Brno, Czech Republic). The daily ration (25 g) contained 20.5 mg of Zn. Group MT rats (tapeworm infected) were also fed ST-1 with added zinc lactate. The amount of ingested food was recorded on a daily basis. Fecal samples were weighed, described and stored in a refrigerator until they were tested in a laboratory for the presence of zinc. The levels of Zn excreted in the feces were calculated on the basis of data regarding the amounts of ingested and excreted zinc.

Analytical procedures

All samples were placed into plastic boxes and stored at -20°C until chemical analysis. Frozen samples were lyophilized and ground into a fine powder (LYOVAC GT 2: LEY-BOLD-HERAEUS, GmbH, Germany). The powder was then added to a mixture of hydrogen peroxide (35 %) and nitric acid (65 %). Finally, the entire mixture underwent microwave digestion.

Concentrations of Cd in the digests were measured by Electrothermal Atomic Absorption Spectrometry using a Varian AA 280Z spectrometer (Varian, Australia) with a graphite tube atomizer GTA 120 and a PSD 120 programmable sample dispenser. Zinc concentrations in digests were determined by optical emission spectroscopy with inductively coupled plasma (ICP-OES) and axial plasma configuration using a Varian VistaPro, equipped with an SPS-5 autosampler (Australia). Measurement conditions for all lines were as follows: 1.2 kW (power); 15.0 L min-1 (plasma flow); 0.75 L min-1 (auxillary flow); and 0.9 L min-1 (nebulizer flow.)

The quality of analytical data was assessed by simultaneous analysis of certified reference material CRM 12-02-01 (Bovine Liver; 4 % of samples). Analytical data obtained for all elements were within the confidence interval given by the producer. The background of the trace element laboratory was monitored by an analysis of 15 % blanks prepared under the same conditions (without samples), and experimental data were corrected by mean concentration of the elements in blanks and compared with detection limits (mean ± 3 SD of blanks) which were 7.5 ng mL-1 for Zn.

Statistical analysis

For each sample, differences between Zn intake and excretion levels were calculated and expressed as a percentage. Statistica 10 software (Statsoft, USA) was used for all computations and statis-

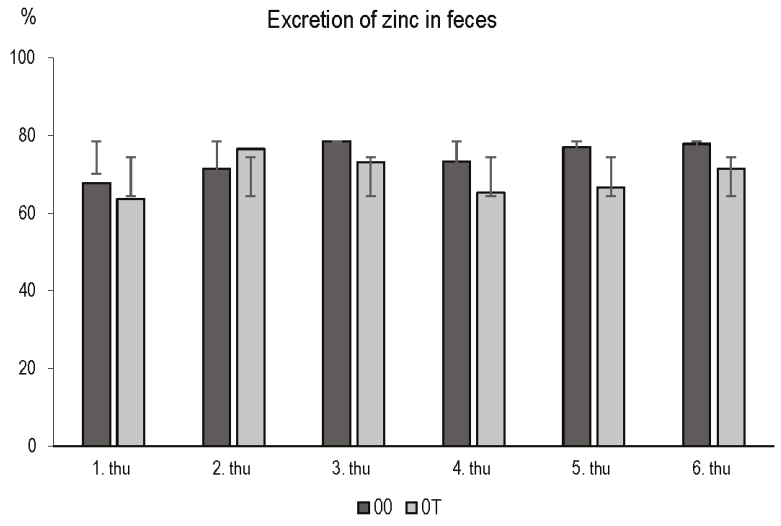

Fig. 1. A comparison of Zn excretion rates (%) in the feces of uninfected control rats (00) and those of control rats (0T) infected with tapeworms (*Hymenolepis diminuta*). thu, Thursday (samples were taken every Thursday)

tical analyses. The normality of data was tested separately using a Shapiro-Wilk test. We then used a nonparametric Mann-Whitney U test to evaluate the proposed hypothesis. Our hypothesis produced a p-value <0.05 (marked with an asterisk in Figure 2) regarding the statistically significant influence of zinc in rat feed. A p-value <0.01 was applied to highlight statistically significant differences, which were indicated with two asterisks in Figure 2.

Ethical Approval and/or Informed Consent

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes [and feed legislation, if appropriate]. All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic, Act No. 246/1992 coll. on the protection of animals against cruelty and EC Directive 86/609 EEC.The study was approved by the ethic committee of Czech University of Life Sciences Prague.

Results

Tapeworms have generally been shown to reduce the amount of zinc in the feces of a host. However, infected rats in the control groups (00 and 0T) excreted more Zn than did rats without tapeworms (6.3 %); this was the case even during the second week of the study. For the remaining weeks, rats in group 0T excreted less Zn in feces than did rats in group 00 (the most dramatic difference was observed in the fifth week: 8.5 %); however, differences between these two groups were not considered significant (Fig. 1). When comparing the groups that are provided with high amounts of zinc in their feed (M0; MT), it is evident that the tapeworm does have an effect on the excretion of this metal in the feces. Throughout the entire six weeks of study, rats of the MT group excreted less zinc in the feces than did uninfected rats (M0). In the first week, only insignificant differences between the two groups were observed. During the next five weeks, however, differences in Zn levels between the two groups were determined to be statistically significant. These differences were evaluated as more significant ($p < 0.01$) in the 3rd (28.4 %), 5th (27 %), and 6th (24.9 %) week (Fig. 2).

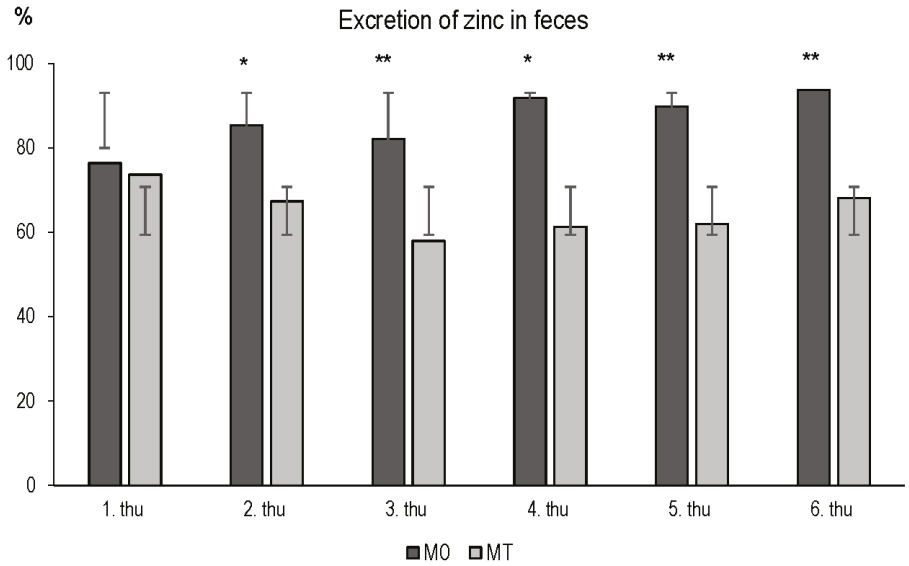

Fig. 2. A comparison between Zn excretion rates (%) in the feces of rats from group M0 (uninfected and fed Zinc lactate) and those of rats from group PT (tapeworm-infected and fed Zinc lactate).
*Statistically significant difference between groups M0 a MT, $p < 0.05$;
**Statistically significant difference between groups M0 a MT, $p < 0.01$;
thu, Thursday (samples were taken every Thursday)

Table 3. Zinc dosages and levels of Zn excreted in feces (mg) over a 6-week period.

Experimental group	Zinc intake (mg/day)	Faecal excretion zinc (mg/day)	Faecal excretion zinc (%)
00	1.75 ± 0.13	1.22 ± 0.18	73.67
0T	1.71 ± 0.16	1.19 ± 0.19	70.19
M0	20.25 ± 1.12	17.64 ± 2.78	87.2
MT	19.23 ± 1.75	12.90 ± 1.79	64.84

Values expressed as the median ± standard deviation in the group

The values of fecal zinc excretion for the entire duration of the experiment indicate that the MT group excreted over 20 % less zinc than did the M0 group. Differences in Zn levels between groups 00 and 0T were not considered significant; rats with tapeworms excreted less zinc in feces than did rats without tapeworms. The M0 group excreted the excreted the highest levels of zinc of all groups, while the MT group excreted the lowest levels. Thus, the difference in zinc levels between groups M0 and MT was significantly greater ($p < 0.01$) than it was between groups 00 and 0T. Tapeworm infection had the strongest effect on zinc levels in the feces of rats fed high doses of zinc (Table 3).

Discussion

Zinc is an element that is essential for the proper functioning of animal metabolism, but it can also be toxic in high doses. Insufficient nutritional intake of zinc is relatively common in some areas, so dietary supplements, among them zinc, are used to complement the diet. These supplements contain various zinc compounds, some of which are well absorbed, while others are not. In our study, we chose zinc lactate as a dietary zinc supplement. Hotz *et al.* (2004), state that in order to focus future research on the intake and excretion of zinc, there is a need to clarify the role gastrointestinal helminths play in these processes. This was the main objective of our study.

Gastrointestinal parasites come into contact with ingested food before it is absorbed by the host's digestive tract, and this has an effect on the amount of nutrients available to the host. Krebs (2000) states that in humans and animals, two factors are most important for maintaining zinc homeostasis - endogenous intestinal excretion and the amount of zinc absorbed by the intestine.

According to Hall *et al.* (2008), tapeworms impair the absorption of nutrients, especially in the final portion of the ileum, of the ileum, and this impairment can lead to anemia. In addition, according to Hotz *et al.* (2004), approximately 30 % of zinc is absorbed in the ileum, and Johnson (1989) reported levels as high as 60 %. Wada *et al.* (1985) report that approximately 53 % and 49 % of zinc is absorbed at dietary doses of 16.5 mg/day and 5.5 mg/day, respectively. In contrast, Jackson *et al.* (1984) state that when dietary zinc is increased, the levels of absorbed zinc decrease. When levels of dietary zinc were doubled, the absorption rate was 45 % for the first 4 days and decreased to 32 % during the following 4-day period. Another study also proves the extraordinary ability of the body to regulate zinc intake. King *et al.* (2000) report that the body absorbs up to 100 % of ingested zinc when a diet is lacking in this metal. When rats were fed 0.5 mg of Zn per day, the absorption rate decreased to 55 % (King *et al.*, 2000).

In our experiment, control rats from groups 0T and 00 received an average of 1.71 mg and 1.75 mg of Zn per day, respectively. Groups 0T and 00 excreted 70 % and 74 % of ingested zinc, respectively. Rats in experimental group M0 excreted, on average, the highest levels of ingested zinc from all the groups, namely 87 % (Table 3); the highest levels (94 %) were eliminated in the final week. This confirms that the organism responds to a higher content of zinc in the diet by excreting higher amounts in the feces.

The study of Cadkova *et al.* (2013) demonstrates how the type of ingested Zn can play an important role in the absorption process. The authors administered lead to rats in two different forms - lead acetate and phytobound Pb (*Pistia stratiotes* (Linné, 1753)). Their results show that 53 % of the ingested lead was excreted in the feces within 24 hours of exposure. In our study, from 65 % (MT group) to 87 % (M0 group) of ingested zinc was excreted 24 hours after exposure.

Cadkova *et al.* (2013) also determined that lead acetate was excreted in greater amounts than lead in hyperaccumulator.

Although zinc was not evaluated in the study of Cadkova *et al.* (2013), we can assume, due to their similar physicochemical properties, that zinc, cadmium and lead (Das *et al.*, 1997; Chaney, 2010), behave in a similar manner. For example, Decker *et al.* (1957) orally administered 2 mg of cadmium to rats. They found that 90 % of the administered dose was excreted in the feces. This amount is similar to the values of zinc excreted by feces. According to a study by Horakova *et al.* (2017), zinc, cadmium and lead are concentrated in the same areas of the tapeworm, namely the immature strobils, so it can be assumed that these metals are indistinguishable from one another for the tapeworm. In our experiment, zinc lactate was used to increase zinc content in feed. Zinc is a compound of metal and organic acid, such as lead acetate.

Based on the results of Cadkova *et al.* (2013), we can expect the amount of zinc excreted in the feces to be higher after the intake of zinc lactate than it would be after the intake of phytobound Zn. This is confirmed by a comparison with the results of one of our previous publications (Sloup *et al.*, 2018), where we evaluated how tapeworms affect the excretion of zinc and cadmium in the

feces of rats fed *Arabidopsis halleri* (Hayek, 1908). The results indicated that the uninfected group that were fed *A. halleri* excreted 78 % and 70 % of zinc and cadmium in the feces, respectively. The group infected with tapeworms and fed *A. halleri* excreted 76 % and 61 % of zinc and cadmium in the feces, respectively. In our new study, rats from the M0 and MT groups excreted 87 % and 65 % of zinc in their feces respectively (Table 3). The levels of zinc excreted in the feces from ingested zinc lactate were actually higher than those from zinc ingested in the form of phytobound Zn. Isaure *et al.* (2015) state that zinc, cadmium and lead are also bound as a metal-organic acid complex in hyperaccumulators, including *P. stratiotes* and *A. halleri*.

This finding is unexpected, as the amount of zinc absorbed and excreted can be affected by phytic acid, which is considered an antinutritional agent that reduces the utilization of certain nutrients, including zinc (House *et al.*, 1982). Moreover, according to Milne *et al.* (1984), phytic acid significantly reduces the absorption of Zn. In contrast, the bioavailability of zinc from zinc lactate is thought to be very high, even higher than that of zinc gluconate, which is very well-absorbed (Shengkui *et al.*, 1994).

The MT group excreted 65 % of ingested zinc, while the infected group that were fed *A. halleri* excreted 76 % of ingested zinc (Sloup *et al.*, 2018). This can be attributed to the ability of the tapeworm to absorb more zinc from a diet rich in zinc lactate, which has a high bioavailability, than it could from a diet with added *A. halleri*. However, it is also possible that the tapeworms of *A. halleri* received less zinc because in addition to Zn, the plants contained hazardous metals, namely cadmium, which the zinc lactate diet did not. Another reason could be tapeworm intoxication in the intestine of rats fed *A. halleri*. According to Scott and Koski (2000), a high zinc content has a negative effect on tapeworms. A relatively high intake of cadmium and zinc could also lead to a poisoning of the tapeworms in cases where rats are fed a diet with added *A. halleri*. This in turn could result in tapeworms with smaller dimensions. These smaller tapeworms would accumulate less zinc than would tapeworms that were exposed to high levels of zinc exclusively through a diet fortified with zinc lactate.

Conclusion

Although the entire complex of processes that ultimately determine how much zinc will be absorbed, excreted, and ultimately accumulated in an animal depends on a number of factors, tapeworms represent an integral and important part of such processes. The amount of metal in the diet is one of the most important factors for homeostasis. However, the type of ingested zinc, food composition and the quantity of other nutrients are also vital. This is evident from our results, which indicated that tapeworm presence had a significant effect on zinc excretion in rats fed high doses of zinc (M0 and MT groups) that was not evident in rats fed a standard diet containing normal amounts of the metal (groups 00 and 0T). Our hypothesis was thus partially confirmed.

Conflict of Interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

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Effects of excessive dietary zinc or zinc/cadmium and tapeworm infection on the biochemical parameters in rats

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Abstract

The main objective of this study was to determine which biochemical blood parameters can serve as indicators of Zn or Zn/Cd burden and tapeworm infection. This study was performed on 44 Wistar male rats during a 6-week period, when rats were or were not fed a zinc/cadmium rich diet and were or were not infected with tapeworms (*Hymenolepis diminuta*). Total protein, albumin, urea, glucose, triacylglycerols, non-esterified fatty acids, cholesterol, alkaline phosphatase, aspartate aminotransferase, uric acid, Mg, Ca, P and Zn levels were analysed. Control rats with tapeworm infection had significantly higher ($p \leq 0.05$) total protein, urea and phosphorus concentrations than did rats unaffected by any experimental factor. Rats given overdoses of zinc lactate exhibited significantly lower glucose levels than did the other rats, especially those infected with tapeworms. Low glucose level in uninfected rats indicate a Zn overdose; high doses of zinc lactate likely decrease levels of glucose via cortisol, which is released during stress. Rats fed the Zn/Cd hyperaccumulating plant *Arabidopsis halleri* and infected with tapeworms had significantly higher ($p \leq 0.01$) cholesterol and urea levels but lower zinc, triacylglycerol, and alkaline phosphatase levels than did rats fed the same diet but free of tapeworms. The increase of alkaline phosphatase level in uninfected rats may indicate both Zn/Cd burden and rat liver damage. Overall, this study not only supports the theory that *H. diminuta* can serve as a promising model for helminth therapy of the host mammal but also confirmed that this tapeworm is capable to protect somehow the host organism from the harmful effects of heavy metals.

KEYWORDS
biochemical parameter, cadmium, rat, tapeworm, zinc

1 | INTRODUCTION

Biochemical parameters are important indicators of the health state of animals and humans and their environment (Dimitrijević et al., 2013; Girling et al., 2015; Shao et al., 2020).

Heavy metal contamination is a major environmental issue that affects the ecology of ecosystems and, consequently, health of live beings. Heavy metals occur through natural sources and anthropogenic emissions of industrial and agricultural origins.

The common rat tapeworm (*Hymenolepis diminuta*, Cestoda, Platyhelminthes) is a common parasite of rodents (including laboratory rodents) but it is known to infect humans as well. For this reason, there is an idea that experimental infection with *H. diminuta* may serve as a promising model for helminth therapy of human immune-mediated inflammatory diseases (IMIDs) because the cestode establishes long-term, stable colonization in hosts and modulates the immune system without causing bacterial dysbiosis (Kosik-Bogacka et al., 2010, 2016; Wegener Parfrey et al., 2017). In previous studies,

it was reported that heavy metal absorption in the digestive tract of hosts is affected by certain intestinal helminths (Sures, 2004); this is primarily true for tapeworms and acanthocephalans, which utilize their metabolically active body surface (tegument) to absorb nutrients (Sures, 2004; Sures & Reimann, 2003). In other studies (Jankovská et al., 2016; Jankovská, Sloup, Szaková, Magdálek, Nechybová, et al., 2018), we worked with a Zn/Cd hyperaccumulating plant (*Arabidopsis halleri*, Brassicaceae) and monitored how its consumption can affect rat organism. The current study aimed to determine the effects of zinc, and to a lesser extent also cadmium (in feed), on the biochemical parameters in laboratory rats infected and/or uninfected with a tapeworm. The objective of this work was to examine possible homeostatic changes in certain biochemical parameters that occur after an organism consumes the Zn/Cd hyperaccumulating plant (*A. halleri*) or is given an overdose of zinc lactate and/or is infected with tapeworms.

In addition to determining the effects of Zn/Cd on the blood biochemical parameters in uninfected rats, this study also focused on changes in these parameters of laboratory rats experimentally infected with rat tapeworm *H. diminuta*. The results of this study may therefore help to determine the possible impacts of these helminths have on a host, and the findings could also lead to the use assessment of the helminths in helminth therapy. *Hymenolepis diminuta* has become a leading candidate for helminth therapy, which is used to treat or prevent inflammatory diseases in humans (Jirků Pomajbíková et al., 2018; Řežábková et al., 2019).

We hypothesize that high doses of Zn or Zn/Cd and tapeworm infection cause a disbalance between certain biochemical parameters which can serve as biological/ecological indicators of an affected environment or as indicators of tapeworm infection.

2 | MATERIALS AND METHODS

2.1 | Design of experiment and laboratory animals

As an experimental animal model, we used 44 males of Wistar laboratory rats (*Rattus norvegicus* var. *alba*) infected (18 rats) or uninfected (26 animals) with the common rat tapeworm (*H. diminuta*). The rats were obtained from a commercial source (Velaz, Czech Republic) and each specimen weighed 150 ± 5 g at the outset of the experiment. Rats were divided into six groups (Table 1): rats from groups 00 and 0T were provided with a commercially manufactured feed (ST-1) and were either uninfected (00) or infected (0T) with the tapeworms; rats in groups M0 and MT were given the ST-1 feed enriched with zinc lactate and also were uninfected (M0)/infected (MT) with the tapeworms; last groups P0 and PT were fed the ST-1 feed enriched the powdered Zn/Cd containing plant *Arabidopsis halleri* and were uninfected (P0)/infected (PT) with the tapeworms.

At the beginning of the experiment, each of 18 rats belonging to groups 0T, MT and PT was infected with three larval cysticercoids of *H. diminuta*. Cysticercoids were acquired from laboratory-bred confused flower beetles (*Tribolium confusum*, Tenebrionidae), which became infected after ingesting tapeworm eggs collected from the excrements of previously infected rats. Cysticercoid development in beetles took place over a 20-day period. The cysticercoids were then collected, suspended in a solution of glucose, and administered to the rats orally via micropipettes (Jankovská et al., 2016). Tapeworm infection was verified by coprological examination which indicated the presence of tapeworm eggs in rat excrements.

The first 3 weeks were intended to acclimatize all rats and develop the mature tapeworms within the infected individuals. At that time, rats were given ad libitum access to both water and a standard ST-1 rodent feed, manufactured by Velaz. The quality contents per 1 kg of ST-1 mixture were as follows: Moisture 12.5%; Nitrogenous substances 24%; Fibre 4.4%; Fats 3.4%; Dry matter 6.0%; Lysin 14 g; Methionin 4.8 g; Calcium (Ca) 11 g; Phosporus (P) 7.2 g; Sodium (Na) 1.8 g; Vitamin A 28,000 I.U.; Vitamin D 2200 I.U.; Vitamin E (Alfatokoferol)—100 mg; Copper (Cu) 20 mg; Selen (Se) 0.38 mg (www.velaz.cz/en/product/st-1/). Animals were placed in a room equipped with an air conditioner and kept at a constant temperature (21–23°C), humidity (approximately 70%) and day–night cycle (08:00–20:00 h).

After the 3 week period when tapeworm infection was verified, each individual rat was placed in its own metabolic cage for next 6-week period. All had unlimited access to water and were fed according to their experimental group.

Whole flowering shoots of the Zn/Cd hyperaccumulating plant *A. halleri* were sampled from area heavily contaminated by Pb, Cd and Zn in the vicinity of Příbram (Czech Republic), dried at laboratory temperature (20°C) and homogenized.

A 25 g daily dose of feed mixture contained 20.5 mg of Zn and 0.41 mg of Cd in groups P0 and PT (*A. halleri*). A 25 g daily dose of a rat from M0 and MT groups contained 20.5 mg of Zn (zink lactate); the rats from groups 00/0T were further fed pure ST-1 feed free from Zn and Cd (www.velaz.cz/en/product/st-1/). All rats received the daily dose only six times per week as they were given no food on Sunday; total weekly doses of heavy metals were 6 × 20.5 mg = 123 mg of zinc and 6 × 0.41 mg = 2.46 mg of cadmium (Table 1).

TABLE 1 Experiment design

Experimental group	Rat number	Zn/week/rat (mg)	Cd/week/rat (mg)	*Hymenolepis diminuta*
00	8	–	–	–
0T	6	–	–	+
M0	12	123	–	–
MT	6	123	–	+
P0	6	123	2.46	–
PT	6	123	2.46	+

Note: Groups 00 and 0T included rats fed a commercially manufactured feed (ST-1), either uninfected (00) or infected (0T) with the tapeworms *H. diminuta*; M0 and MT rats were fed (ST-1) feed enriched with zinc lactate, either uninfected (M0) or infected (MT) with the tapeworms; P0 and PT rats were fed (ST-1) feed enriched with the powdered Zn/Cd containing plant *Arabidopsis halleri*, either uninfected (P0) or infected (PT) with the tapeworms.

2.2 | Biochemical analysis

We collected blood of rats immediately after administering an overdose of anesthetic (chloroform inhalation) through the *cavum abdominis* veins. The samples were then coagulated at laboratory temperature and later centrifuged at 1000 *g* for 15 min. The separated serum was kept in a deep freeze (−80°C) until it was taken for analysis. We looked at the following fourteen biochemical parameters from the blood (serum) samples: total protein (TP), albumin (ALB), urea (UREA), glucose (GLU), triacylglycerols (TG), non-esterified fatty acids (NEFA), cholesterol (CHOL), alkaline phosphatase (ALP), aspartate aminotransferase (AST), uric acid (UA), magnesium (Mg), calcium (Ca), phosphorus (P) and zinc (Zn). We identified zinc using a manual method that was carried out with a spectrophotometer (Libra S6; Biochrom). We used a Randox Zinc commercial kit (Randox Laboratories) to evaluate colorimetric method performance. Using an ERBA XL 200 automatic analyzer (Erba Diagnostics Mannheim GmbH), we spectrophotometrically identified the remaining blood parameters in the laboratory of the Czech University of Life Sciences Prague. Analysis was carried out using commercial kits, namely from Randox NEFA (Randox Laboratories), as well as various other kits manufactured by Erba Diagnostics Mannheim GmbH.

2.3 | Data analysis

We used a nonparametric Mann–Whitney *U*-test to evaluate the proposed hypothesis concerning possible influence of tapeworm infection or heavy metal (zinc/cadmium) in rat food on some blood biochemical parameters in rats. The **p*-value ≤ 0.05; ***p*-value ≤ 0.01 indicated statistically significant differences and they were marked with asterisks in Figures 1–3. Statistics software STATISTICA 10 (Statsoft) was used for all computations and statistical analysis.

3 | RESULTS

3.1 | Effect of tapeworm infection itself

Monitored biochemical parameters of rats from the control group (00) were considered physiological values as these rats were not affected by any experimental factor. As it is shown in Table 2 and Figure 1, control group (00) had an average level of total protein of 52.15 g/L (physiological value) while this value was significantly higher ($p \leq 0.05$) in rats with tapeworm infection (0T). Similar trend was evident in the other experimental groups (M0/MT and P0/PT), but the differences were not statistically confirmed (Table 2,

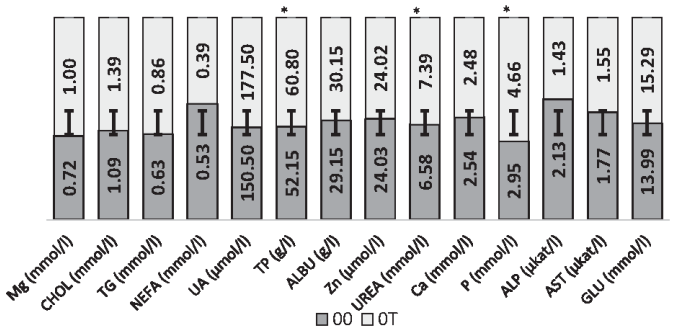

FIGURE 1 Biochemical parameters of rats (group 00 = control/non-affected rats; group 0T = rats infected with tapeworms). **p*-value ≤ 0.05

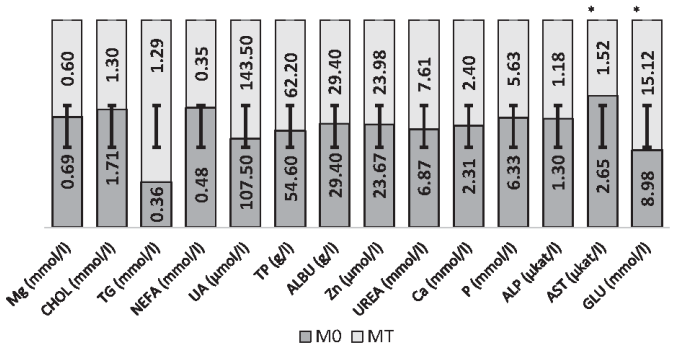

FIGURE 2 Biochemical parameters of rats (group M0 = rats overdosed with zinc lactate; group MT = rats overdosed with zinc lactate and infected with tapeworms). **p*-value ≤ 0.05

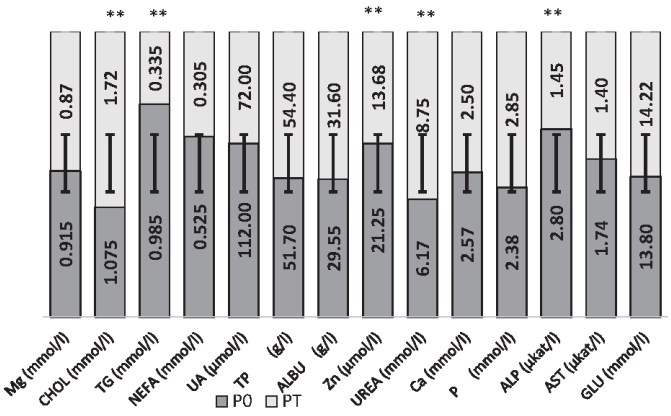

FIGURE 3 Biochemical parameters of rats (groups P0 = rats given Zn and Cd hyperaccumulating plant [*Arabidopsis halleri*] powder in feed mixture; PT = rats given Zn and Cd hyperaccumulating plant [*A. halleri*] powder in feed mixture and infected with tapeworms). $^{**}p$-value ≤ 0.01

TABLE 2 Summary of biochemical parameters of rats from six experimental groups

		Experimental group					
Biochemical parameter	Short cut (value)	00	0T	M0	MT	P0	PT
Magnesium	Mg (mmol/L)	0.72	1.01	0.69	0.60	0.92	0.87
Cholesterol	CHOL (mmol/L)	1.09	1.39	1.71	1.30	1.08	1.72**
Triacylglycerols	TG (mmol/L)	0.63	0.86	0.36	1.29	0.99	0.34**
Non-esterified fatty acid	NEFA (mmol/L)	0.53	0.39	0.48	0.35	0.53	0.31
Uric acid	UA (µmol/L)	150.50	177.50	107.50	143.50	112.00	72.00
Total protein	TP (g/L)	52.15	60.80*	54.60	62.20	51.70	54.40
Albumin	ALB (g/L)	29.15	30.15	29.40	29.40	29.55	31.60
Zinc	Zn (µmol/L)	24.03	24.02	23.67	23.98	21.25	13.68**
Urea	UREA (µmol/L)	6.58	7.40*	6.87	7.61	6.18	8.75**
Calcium	Ca (µmol/L)	2.54	2.48	2.31	2.40	2.57	2.50
Phosphorus	P (µmol/L)	2.95	4.67*	6.33	5.64	2.38	2.85
Alkaline phosphatase	ALP (µkat/L)	2.13	1.44	1.30	1.18	2.80	1.45**
Aspartate aminotransferase	AST (µkat/L)	1.77	1.55	2.65	1.53*	1.74	1.40
Glucose	GLU (mmol/L)	13.99	15.29	8.98	15.12*	13.80	14.22

Note: Control rats – 00; control rats infected with tapeworms – 0T; rats fed high doses of zinc lactate – M0; rats fed high doses of zinc lactate and infected with tapeworms – MT; rats fed Zn and Cd hyperaccumulating plants – P0; rats fed Zn and Cd hyperaccumulating plants and infected with tapeworms – PT.

*p-value ≤ 0.05.

**p-value ≤ 0.01.

Figures 2 and 3). Rats infected with tapeworms (0T) had also significantly higher ($p \leq 0.05$) urea and phosphorus levels compared to uninfected control rats (00).

3.2 | Effect of zinc lactate and tapeworm infection

Rats given overdoses of zinc lactate without tapeworm infection (M0) had significantly lower ($p \leq 0.05$) glucose levels (8.98 mmol/L) than rats from all other experimental groups including this with zinc lactate overdose and tapeworm infection (MT) which was statistically confirmed (Table 2, Figures 1–3). Another significant difference ($p \leq 0.05$) was found in the case of aspartate aminotransferase level, which was increased only in uninfected rats fed zinc lactate (Table 2, Figure 2).

3.3 | Effect of Zn/Cd hyperaccumulating plant (*A. halleri*) and tapeworm infection

The apparent effect of the zinc and cadmium dose contained in the *A. halleri* plant (group P0), in combination with the tapeworm

infection (group PT), was manifested in up to five analysed parameters (Table 2, Figure 3). First, significant difference ($p \leq 0.01$) was found in mean cholesterol levels which were higher (1.72 mmol/L) in infected rats (PT) compared with uninfected rodents (P0), all fed Cd/Zn hyperaccumulating plant (Table 2, Figure 3). Further, tapeworm infection significantly decreased ($p \leq 0.01$) triacylglycerol, alkaline phosphatase and zinc levels in the blood serum of rats fed *A. halleri*, but this effect has not been proven in the other groups (Table 2, Figures 1 and 2). Contrariwise, rats that fed *A. halleri* and infected with tapeworms (PT) had significantly higher urea level ($p \leq 0.01$) in blood than did rats without tapeworm infection (P0), and this trend was slightly apparent everywhere (Table 2).

4 | DISCUSSION

This study expands our previous knowledge of the effects of Zn/Cd hyperaccumulating plant *A. halleri* on rat organism (Jankovská et al., 2016; Jankovská, Sloup, Szaková, Magdálek, Nechybová, et al., 2018) and examines effects of new factors influencing the physiology of these experimental animals. These factors are the overdose of zinc lactate in the diet, and the combination of various diets with infection with the rat tapeworm *H. diminuta*. This gut parasite is considered suitable for helminth therapy (Kosik-Bogacka et al., 2010, 2016; Řežábková et al., 2019; Wegener Parfrey et al., 2017). First, we compared changes in various biochemical parameters in uninfected and host rats, both fed a standard feed (groups 00 and 0T). Only total protein, urea and phosphorus levels were significantly higher in the serum of rats with tapeworm infection than in rats of the unaffected control group. Other parameters (albumin, glucose, triacylglycerols, non-esterified fatty acids, cholesterol, alkaline phosphatase, aspartate aminotransferase, uric acid, magnesium, calcium and zinc) were not significantly affected by tapeworm infection. This supports the findings of Wegener Parfrey et al. (2017) that *H. diminuta* is a benign intestinal probiotic helminth, which can be used in helminth therapy. *Hymenolepis diminuta* is thus a promising model for helminth therapy because it establishes long term, stable colonization in rats and modulates the immune system without causing bacterial dysbiosis. Significantly higher total protein levels of rats with tapeworm infection (0T) can be caused by any condition that creates an increase in immunoglobulins, this finding can also be utilized in helminth therapy to treat autoimmune diseases due to tapeworm potent immunoregulatory properties (Wangchuk et al., 2019).

Numerous studies have shown that treatment with certain doses of a zinc supplement in cadmium intoxicated animals decreases cadmium absorption and accumulation, and reduces some of its toxic effects (Rogalska et al., 2009). Cadmium and zinc are closely related metals with similar chemical properties. However, unlike cadmium, zinc is an essential element needed to activate many enzymes and hormones (Hejazy & Koohi, 2017). *Arabidopsis halleri* plant accumulates both zinc and cadmium, so we can therefore assume that the negative influence of cadmium partially counterbalances the presence of zinc. However, Hejazy and Koohi (2017) monitored the effects of nano-zinc on the biochemical parameters of cadmium exposed rats, and their study indicated that nano-zinc particles do not have any protective effects against cadmium toxicity; moreover, the study further revealed the toxic effects of orally administered nano-zinc particles.

Regardless of zinc intake, zinc concentrations in blood plasma do not fluctuate dramatically (Whitney & Rolfes, 2013). This seems to support the study findings, which indicate that zinc lactate overdosing (M0) and Zn/Cd hyperaccumulating plants (P0) had no effect on zinc levels in rat serum. However, Zn/Cd hyperaccumulating plants and tapeworm infection (PT) significantly decreased zinc levels in rat serum. This may be due to the ability of tapeworms to accumulate heavy metals (especially elements in excess) from the host body (Jankovská et al., 2016; Jankovská, Sloup, Szaková, Magdálek, Horáková, et al., 2018). However, for rats given an overdose of zinc lactate (M0, MT), tapeworm infection did not decrease zinc levels in their blood. This phenomenon can be affected by the element form, which is received by host; the tapeworms can then accumulate the element and subsequently reduce levels in the host tissues.

In our experiment, the lowest urea concentrations were seen in rats fed Zn/Cd hyperaccumulating plants and not infected with tapeworms (P0), followed by rats given high doses of zinc lactate in feed (M0). Low blood urea concentrations can be a result of a low protein diet or liver damage, which can be caused by the Cd in the feed mixture (group P0). High blood urea concentrations can be caused by poor renal function, kidney damage, a high protein diet, protein catabolism or dehydration (Hejazy & Koohi, 2017). In our experiment, the highest urea concentrations were found in rats fed Zn/Cd hyperaccumulating plants and infected with tapeworms (PT group). Tapeworm infection (groups PT, MT, 0T) generally increased urea concentrations in parasitized rats. Dimitrijević et al. (2013) also reported that serum urea levels in sheep infected with rhabditid nematodes (*Strongyloides papillosus*) were higher than those in untreated and uninfected sheep. Therefore, it is possible that nematode infection can also increase serum urea levels in a host.

Alkaline phosphatase (ALP) is considered cholestatic-induction enzyme of hepatobiliary origin (Ramaiah, 2007). In our experiment, the mean physiological ALP value in control unaffected rats (group 00) was 2.13 μkat/L; the highest ALP level (2.80 μkat/L) was seen in rats from group P0 (Zn/Cd hyperaccumulating plant). There was a significant difference between ALP levels in rats from group PT (1.45 μkat/L) and those from group P0 (2.80 μkat/L); Kaslow (2014) reported that ALP activity can be hindered by low zinc levels. Zinc deficiency can be associated with tapeworm infection and the ability of tapeworms to accumulate zinc (Jankovská et al., 2016).

Aspartate aminotransferase (AST) is commonly used in clinical settings as a marker for liver health. The reversible transfer of α-amino groups between aspartate and glutamate is catalysed by AST, and so it is a vital enzyme in the metabolism of amino

acids. AST can be observed in the liver, heart, skeletal muscle, kidneys, brain and red blood cells. The AST test is commonly used to detect potential liver damage (Zhang et al., 2004). Hejazy and Koohi (2017) reported that levels of aspartate aminotransferase, alanine aminotransferase, triglycerides, total cholesterol and free fatty acids increased significantly in the cadmium and nano-zinc-treated rats compared with those of the control groups. In our experiments, control rats (group 00) had a physiological AST level of 1.77 μkat/L. AST levels (2.65 μkat/L) in rats given an overdose of zinc lactate (M0) were significantly higher than those in rats given an overdose of zinc lactate and infected with tapeworms (MT, 1.53 μkat/L), therefore, tapeworm infection in rats can decrease AST levels through the accumulation of excess zinc into the tissues of the tapeworm (Jankovská, Sloup, Szaková, Magdálek, Horáková, et al., 2018).

In accordance with Hejazy and Koohi (2017), cholesterol level in our experiments was the highest (1.72 mmol/L) in rats given cadmium and zinc hyperaccumulating plants and infected with tapeworm (PT). Rath and Walkey (1987) described that tapeworm infection increased the rates of cholesterol synthesis in mice. It is interesting why the cholesterol level was similarly high (1.71 mmol/L) in rats given an overdose of zinc lactate without tapeworm infection (M0).

5 | CONCLUSION

The infection of rats with the tapeworm *H. diminuta* exhibited significant effect on host biochemical parameters only in total protein, urea and phosphorus. Zinc lactate overdose affected only the glucose and aspartate aminotransferase level of consumer. Low glucose levels may indicate a Zn overdose; we concluded that high doses of zinc lactate can help decrease levels of glucose caused by cortisol, which is released during stress. However, Zn/Cd in hyperaccumulating plant increased primarily cholestatic-induction enzyme of hepatobiliary origin (alkaline phosphatase) and decreased zinc concentration in the consumer mammal; it can be caused by tapeworm ability to absorb zinc in their tissue/body.

Our hypothesis was confirmed primarily for effect of Zn/Cd in hyperaccumulating plant because high doses of Zn/Cd cause a disbalance of five biochemical parameters (cholesterol, triacylglycerols, zinc, urea and alkaline phosphatase) which can serve as biological/ecological indicators of an affected environment.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic, Act No. 246/1992 coll. on the protection of animals against cruelty and EC Directive 86/609 EEC. The study was approved by the ethic committee of Czech University of Life Sciences Prague.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Significance of Intestinal Helminth Infection and Animal Sex for Mercury Concentrations in Two Rodent Species

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ABSTRACT: We compared the effects of animal gender, species, and intestinal helminth burden on mercury concentrations in rodents. Total mercury concentrations were determined in the liver and kidney tissues of 80 small rodents (44 yellow-necked mice, *Apodemus flavicollis*, and 36 bank voles, *Myodes glareolus*) captured in the Ore Mountains (northwest Bohemia, Czech Republic). Overall, 25/80 (32%) of animals were infected by intestinal helminths. The differences in mercury concentration between rodents infected and not infected with intestinal helminths were not statistically significant. Statistically significant differences in mercury concentrations were found only between voles and mice (that were not infected with intestinal helminths). This suggests the differences may be associated with host genetics. *Apodemus flavicollis* body tissues had significantly lower (P=0.01) mean Hg concentrations (0.032 mg/kg) than *Myodes glareolus* (0.279 mg/kg), provided that animals were not infected by intestinal helminths; if the animals were infected by intestinal helminths, the difference between both groups was insignificant. The effect of gender in this study was significant only for voles (without helminth infection); for mice (either with or without helminth infection) the differences between genders were not significant. *Myodes glareolus* males had significantly lower (P=0.03) Hg concentrations in liver and kidney tissues (0.050 mg/kg) than *Myodes glareolus* females (0.122 mg/kg). These results reveal the importance of considering species and gender when evaluating mercury concentrations.

Key words: Accumulation, helminth, host, mercury, sex, tissue.

Environmental heavy metal pollution is a global, long-term problem (Kooyomjian et al. 2022; Palmer et al. 2022). The heavy metal mercury is primarily emitted from industrial sources, especially coal burning. Mercury contamination of the environment is also a persistent problem from former industrial and agricultural activities (Brázova et al. 2021). Mercury is a significant pollutant that is highly toxic, and relatively small concentrations can adversely affect the reproduction, development, growth, metabolism, behavior, and immune responses of animals (Rhea et al. 2013; Saavedra et al. 2022). Negative effects of mercury on reproduction include an increased risk of reduced fertility, spontaneous abortion, and congenital deficits or abnormalities (Bjørklund et al. 2019).

The bioconcentration and accumulation mechanisms of mercury are not completely understood. There is scant knowledge of the mercury levels in terrestrial mammals, even though they are routinely used in the biomonitoring of different environmental contaminants (Durkalec et al. 2019). The study aimed to determine the effect of intestinal helminths and the sex of small mammals (rodents) on the mercury concentration in their tissues. We hypothesized that 1) intestinal helminth presence influences mercury concentration in the host tissues and 2) the sex of the animal affects the concentration of mercury in their body.

In autumn 2019, we caught 80 small rodents (yellow-necked mice, *Apodemus flavicollis*, and bank voles, *Myodes glareolus*) from the Ore Mountains (northwest Bohemia, Czech Republic; Fig. 1). We used snapping traps baited with a roasted wick (soaked in fat with flour); the trapped animals were killed immediately when the traps were closed (by breaking the cervical spine). Rodents were frozen immediately after collection. In the laboratory, the animals were dissected, intestinal helminths were removed, and tissue

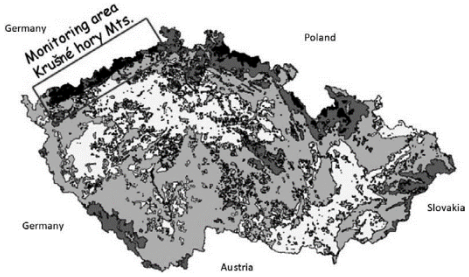

FIGURE 1. Map of the Czech Republic showing the area in the Ore Mountains, northwest Bohemia, where *Myodes glareolus* and *Apodemus flavicolis* were collected during fall 2019 for determination of mercury concentrations.

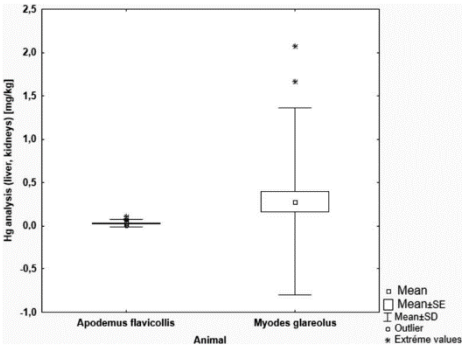

FIGURE 2. Mean mercury concentrations with standard error (SE, box), standard deviation (SD, whiskers) and outlier values for *A. flavicollis* and *M. glareolus* without intestinal helminths collected fall 2019 in the Ore Mountains, northwest Bohemia, Czech Republic.

samples were collected. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. Treatment of animals was conducted in compliance with the current laws of the Czech Republic, under Section 15 d (3) of Act No. 246/1992 Collection of Laws, on the Protection of Animals against Cruelty, and Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.

Tissue samples were collected using stainless steel scissors and forceps cleaned with redistilled water; then frozen in polypropylene containers until further processing. The samples were lyophilized and homogenized, then 20 mg of this homogenized sample was weighed and placed in an advanced mercury analyzer (AMA 254; Altec s.r.o., Czech Republic), a single-purpose atomic absorption spectrometer for mercury determination developed and produced in the Czech Republic. The analyzer incorporates a mercury vapor generation technique and does not require sample chemical pretreatment (e.g., mineralization). The accuracy of the measured data was verified using a certified reference material, ERM-BB186 pig kidney (European Commission, Joint Research Centre JRC, Institute for Reference Materials and Measurements [IRMM], Geel, Belgium). Each sample was measured in duplicate.

The values obtained were initially processed using Excel software (Microsoft, Redmond, Washington, USA). The software program TIBCO Statistica, version 12 CZ (TIBCO Software, Palo Alto, California, USA) was used for statistical data analysis and for creating the graphs. The data were expressed as means ± SD (standard deviation). One-way between groups analysis of variance (ANOVA) was then used (ANOVA is equivalent to Levene's test, a test of homogeneity variance). Tukey's HSD post hoc test was chosen for post hoc analysis to detect differences between individual categories. The Tukey test tests all differences between means with experimental risk of type α_e (risk of the second type is still pairwise; Rasch et al. 1999). A 95% confidence interval was selected. Values for median, standard error, outlier, and extreme values were used as auxiliary indications.

Overall, 31% (25/80) of animals were infected by intestinal helminths; the majority of animals (80%, 20/25) were infected by nematodes (Heligmosomidae); tapeworms (*Hymenolepis* spp.) were present only in *A. flavicollis*. Tapeworms were pooled into one sample, the mean Hg concentration in tapeworms being 0.035 mg/kg. We found that

TABLE 1. Mean mercury concentration ± standard deviation (mg/kg) in *Myodes glareolus* and *Apodemus flavicolis* from the Ore Mountains, northwest Bohemia, Czech Republic, 2019.

	Without helminths (mg/kg)	With helminths (mg/kg)
Myodes glareolus		
Total (n=36)	0.279±0.54* (n=21)	0.078±0.05 (n=15)
Male (n=13)	0.050±0.02** (n=6)	0.051±0.02 (n=7)
Female (n=23)	0.351±0.61** (n=15)	0.097±0.06 (n=8)
Apodemus flavicolis		
Total (n=44)	0.032±0.02* (n=33)	0.025±0.01 (n=11)
Male (n=20)	0.034±0.02 (n=15)	0.029±0.01 (n=5)
Female (n=24)	0.031±0.02 (n=18)	0.021±0.01 (n=6)

* P=0.01, ** P=0.03, statistically significant differences.

intestinal helminths were present in rodents with a lower mercury concentration (0.055 mg/kg) and were not present in rodents with higher Hg tissue concentrations (0.062 mg/kg); however, the difference was not significant (P=0.32).

We found a difference in tissue Hg concentration between species in individuals not infected with intestinal helminths: *A. flavicollis* body tissues showed significantly lower (P=0.01) mean Hg concentrations (0.032 mg/kg) than *M. glareolus* (0.279 mg/kg; Fig. 2; Supplementary Material Table S1). In contrast, in animals infected by helminths, the difference between the species was not significant (Table 1).

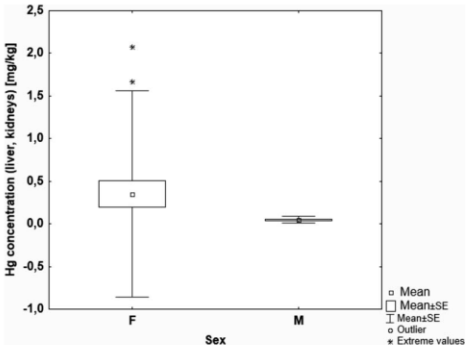

FIGURE 3. Mean mercury concentrations with standard error (SE, box), standard deviation (SD, whiskers) and outlier values in male and female *Myodes glareolus* collected fall 2019 in the Ore Mountains, northwest Bohemia, Czech Republic.

We found a significant effect of sex on mercury concentration in animal tissues in *M. glareolus* (Fig. 3; Supplementary Material Table S2). Females of this species showed significantly (P=0.03) higher (0.122 mg/kg) mean tissue Hg concentrations than males (0.050 mg/kg). No significant difference (P=0.56) in mercury concentration depending on sex was found between *A. flavicollis* females (0.029 mg/kg) and males (0.033 mg/kg).

A previous study found that the presence of the intestinal helminth *Acanthocephalus lucii* also was not associated with reduced mercury concentrations in host fish muscle and gonads (Jankovská et al. 2012); conversely, other studies have found decreased host concentrations of heavy metals such as lead and zinc in acanthocephalans and tapeworm-infected, compared to uninfected, animals (Jankovská et al. 2010a, b, 2011, 2018).

Mercury in organisms may increase helminth prevalence via impairment of the host's immunity. Krupicer (1995) described increased helminth prevalence and intensity of infection in sheep from the control site to the nearest Hg emission source. Mercury levels in host tissues and intestinal contents are probably altered by the presence of intestinal helminths; consequently, the bioavailability of mercury to the host may also be altered (McGrew et al. 2018). Contrariwise, the internal environment of the Hg-affected host may be unfavorable for intestinal helminths, that is, mercury may decrease helminth infection via toxicity effects. In the environ-

ment, the impact of Hg on parasite survival was monitored by Pietrock et al. (2001), who found a significant negative correlation between mean survival times of free-living stages of *Diplostomum* sp. and sediment Hg content.

Our results identified differences between tissue mercury concentrations between *A. flavicollis* and *M. glareolus* unless they were infected by intestinal helminths. *A. flavicollis* and *M. glareolus* are the most common free-living rodents used in heavy metal biomonitoring. Our findings indicate that it is important to use animals without intestinal helminths for such studies.

Intestinal helminth infections affect several parameters in the host including biochemical parameters (Sloup et al. 2021). Intestinal helminth infections may cause immunological responses and pathophysiological changes in their hosts. Kosik-Bogacka et al. (2010) reported that, due to a close relationship between the local immune system and epithelial cells in the gastrointestinal tract, local immunoreactions could directly change the epithelial ion transport causing an increased secretion, decreased ion absorption, or both. This may explain why the presence of intestinal helminths in our study was associated with a change in the concentration of Hg in the host tissue, and we found differences in the concentration of Hg in rodents infected and uninfected by helminths.

Additionally, our study focused on the effect of the animal sex on the mercury concentration in body tissues. Previous work has found that fish gender was relevant in environmental mercury monitoring, because perch male gonads accumulated significantly more Hg than perch female gonads (Jankovská et al. 2014). Similarly, Provencher et al. (2016) reported significantly higher Hg concentrations in Northern Common Eider (*Somateria molissima borealis*) males than females; the authors state that it may be related to the birds' body condition and liver mass. Studies of methylmercury exposed mice showed higher overall mercury retention in females than in males, while the relative kidney deposition was twice as high in males than in females (Nielsen and Andersen 1991; Vahter et al. 2007). Komov et al. (2017) detected 0.021 and 0.014 mg Hg/kg dry weight in the kidneys and liver, respectively, of *M. glareolus*, which was less than we found in *M. glareolus* liver and kidney. However, Komov et al. (2017) reported neither the helminth load nor the sex of examined animals. In our current study, we found a significant ($P<0.05$) effect of sex on mercury concentration in liver and kidney tissues in *M. glareolus* (Arvicolinae), being higher in females, but we did not find any difference by sex in *A. flavicollis*. Mercury is an endocrine disruptor (Milton 2010), and mercury-associated polycystic ovary syndrome has been described in rats (Merlo et al. 2019). In the case of free-living rodents, it is possible that environmental mercury may contribute to decreasing the female fecundity of overpopulated animals.

The differences found in this study may be related to host genetics, the activity of some genes of individual species, and their food preference. For these reasons, it would be appropriate, especially for biomonitoring, to always distinguish between the specific animal species sampled and their parasite infection status, especially gastrointestinal helminth presence, as well as sex.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-22-00129.

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1) Jankovská I, Sloup V, Száková J, Magdálek J, Nechybová S, Peřinková P, Langrová I. 2018. How tapeworm infection and consumption of a Cd and Zn hyperaccumulating plant may affect Cu, Fe, and Mn concentrations in an animal—a plant consumer and tapeworm host. Environ Sci Pollut Res **25**:4190–4196. https://doi.org/10.1007/s11356-017-0787-3 (**Q2 dle AIS**)

2) Jankovská I, Sloup V, Száková J, Langrová I, Sloup S. 2016. How the tapeworm *Hymenolepis diminuta* affects zinc and cadmium accumulation in a host fed a hyperaccumulating plant (*Arabidopsis halleri*). Environ Sci Pollut Res **23**:19126–19133. https://doi.org/10.1007/s11356-016-7123-1 (**Q2 dle AIS**)

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RESEARCH ARTICLE

How tapeworm infection and consumption of a Cd and Zn hyperaccumulating plant may affect Cu, Fe, and Mn concentrations in an animal—a plant consumer and tapeworm host

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Abstract

This study evaluated the effects of a hyperaccumulator plant (*Arabidopsis halleri*), containing surplus of cadmium (Cd) and zinc (Zn) and being an admixture to the rat feed, on concentrations of copper (Cu), iron (Fe), and manganese (Mn) in the tissues of experimental rats infected/uninfected with the tapeworm (*Hymenolepis diminuta*). Male Wistar rats were divided into three groups (00, P0, and PT); the P0 and PT animals were fed a standard mixture for rats (ST-1) supplemented with the plant *A. halleri* at a weekly Zn and Cd dosage of 123 and 1 mg, respectively. Moreover, rats from the group PT were infected with the tapeworm. The group 00 served as control animals fed only ST-1 having no tapeworm infection. Rats were euthanized after 6 weeks, and Cu, Fe, and Mn levels were determined in rat and tapeworm tissues. The results indicated that both the consumption of hyperaccumulator plant and/or presence of tapeworms did have significant effect on Cu, Fe, and Mn concentrations in the host tissues. Concentrations of all the elements were higher in the rat liver and partially kidneys than in the tapeworms, and the concentrations of Cu, Fe, and Mn were affected by the consumption of Cd/Zn hyperaccumulator plants. Particularly, Fe concentrations in all rat tissues were significantly increased by consumption of *A. halleri* while decreased by the presence of tapeworms. Overall, the consumption of a Cd/Zn hyperaccumulator plant and tapeworm infection cause an imbalance in Cu, Fe, and Mn concentrations in the tissues of a consumer (experimental rats).

Keywords Rat · Tapeworm · Plant · Accumulate · Cadmium · Zinc · Copper · Iron · Manganese

Introduction

Optimal nutrition support is critical for mammalian development and health (Collins et al. 2010; Sharma et al. 2016). Zinc, copper, chromium, manganese, and cobalt are essential trace elements. These are defined as elements contained in low concentrations (mg kg^{-1} or less) in plants and animals (Phipps 1981) and are essential for metabolic processes in animals but may be toxic at higher levels. Biogenic (Cu, Cr, Co, Mn, Zn) and toxic (Pb, Cd, Hg) elements in soil can be released (under suitable physicochemical conditions) and be made potentially available for plants in areas with elevated levels of these elements. Soil-plant transfer can therefore serve as a possible means by which these metals become part of the food chain (Čadková et al. 2013). Exposure to low doses of toxic metals affects the homeostasis of toxic and essential metals in animal as well as human tissues. In animal organisms, transport of metals takes place in the bloodstream, which distributes them to various body tissues (liver, kidney, brain, lung, spleen, bone, and teeth). These metals can accumulate to highly toxic levels and have serious negative impacts on living organisms (Sinicropi et al. 2010; Jankovská et al. 2014; Adel et al. 2017).

Interactions between toxic metals and essential bioelements take place in an organism. One significant problem is the interaction between Cd and other mineral nutrients

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that are antagonistic to Cd absorption (Reeves and Chaney 2008). Certain plants, including *Arabidopsis halleri*, can tolerate or even accumulate very high concentrations of cadmium and zinc. Such plants are known as hyperaccumulators and can considerably accelerate the introduction of soil-bound toxic elements into the food chain. Moreover, these species can also be used in phytoremediation and heavy metal monitoring in the environment (Zhenli et al. 2005).

The majority of elements found in animals enter through the oral route and are subsequently absorbed in the digestive tract. This absorption process significantly interferes with the gastrointestinal parasites. This is especially true for acanthocephalans and also tapeworms, which receive nutrients through the tegument, a metabolically active body surface (Sures et al. 2000a, b, 2002; Eira et al. 2005; Kosik-Bogacka et al. 2010). Tapeworms are able to accumulate considerable amounts of metals, thereby reducing their concentrations in host tissues (Jankovská et al. 2010a, b; Čadková et al. 2013; Brožová et al. 2015).

There is little information regarding the interactions of low dose toxic metal mixtures with essential metals. Cobbina et al. (2015) reported that exposure to low doses of metal mixtures (Pb, Hg, As, and Cd) affected the homeostasis of toxic and essential metals in tissues of mice.

Our previous study (Jankovská et al. 2016) showed significantly lower Zn and Cd concentrations in rats infected with the tapeworms compared to the uninfected rats. However, the potential effect of the shifts in Cd and Zn utilization can lead to the changes in uptake and utilization of the important essential elements. These aspects are not fully understood. The aim of the present study was to investigate the ability of the rat tapeworm *Hymenolepis diminuta* to accumulate not only zinc and cadmium (previously reported by Jankovská et al. 2016) but also Cu, Fe, and Mn. Moreover, the experiments made clear whether the consumption of a Cd/Zn hyperaccumulator plant and tapeworm infection cause an imbalance in Cu, Fe, and Mn concentrations in the tissues of a consumer (experimental rats).

Material and methods

The experimental procedures, experimental design, and analytical procedures are described in detail in a previously published study (Jankovská et al., 2016). In this paper, male Wistar rats (*Rattus norvegicus* var. *alba*) were divided into three groups of six individuals (00, P0, and PT). Rats from groups (P0 and PT animals) were fed a standard mixture for rats (ST-1) supplemented with the hyperaccumulating plant *A. halleri* at a weekly Zn and Cd dosage of 123 and 1 mg, respectively. Moreover, rats from the group PT were infected with a rat tapeworm (*H. diminuta*). Rats from 00 group served as control animals, fed only ST-1, and were not infected with the tapeworm infection. Rats were euthanized after 6 weeks, and Cu, Fe, and Mn levels were determined in both rat and tapeworm tissues. The composition of the feeding mixture (Table 1) and experimental design (Table 2) are presented here. In contrast to elevated Cd and Zn levels found in *A. halleri*, the Cu, Fe, and Mn levels did not show an increase compared to common plant values of these elements. For instance, (Blum et al. 2009) presented the normal essential element contents in plants in the range 3.0–12 mg/kg, 50–200 mg/kg, and 20–400 mg/kg for Cu, Fe, and Mn, respectively.

Statistical analysis

We compared element concentrations between the three groups (00, P0, PT) using the Kruskal-Wallis and Mann-Whitney tests. Statistica 10 software (Statsoft, USA) was used for all computations.

Results and discussion

The interaction of Cd and Zn with other essential metals (Cu, Fe, Mn) is not fully understood. We monitored the effects of tapeworm infection (*H. diminuta*) in combination with elevated uptake of Zn and Cd on levels of Cu, Fe, and Mn in the tissues of laboratory rats.

Since 1990s, the ability of various intestinal helminths (primarily acanthocephalans in fish) to accumulate considerable concentrations of heavy metals was described (Lafferty 1997; Sures et al. 1998; Sures 2001; Sures and Siddall 2001; Sures 2003; Thielen et al. 2004) including the decreasing element concentrations in a host. Those parasites can therefore serve as sentinel organisms for heavy metal environmental pollution monitoring; the benefits to the host, however, remain

Table 1 Composition of ST-1 (commercially available from Velaz Ltd. CR)

Moisture	%	12.5
Nitrogen compounds	%	24
Fiber	%	4.4
Lipids	%	3.4
Ash	%	6.8
Lysin	mg/kg	14,000
Methionine	mg/kg	4800
Ca	mg/kg	11,000
P	mg/kg	7200
Na	mg/kg	1800
Cu	mg/kg	20
Zn	mg/kg	70
Se	mg/kg	0.38

Table 2 Experiment design

Group	*n* of animals	Zn/week (mg)	Cd/week (mg)	Tapeworm *H. diminuta*
Plant (P0)	6	123	1	–
Plant and tapeworm (PT)	6	123	1	+
Control (00)	6	10.5	0	–

inconsistent (Sures et al. 2002; Baruš et al. 2003; Torres et al. 2004, 2006; Jankovská et al. 2008, 2009, 2010c).

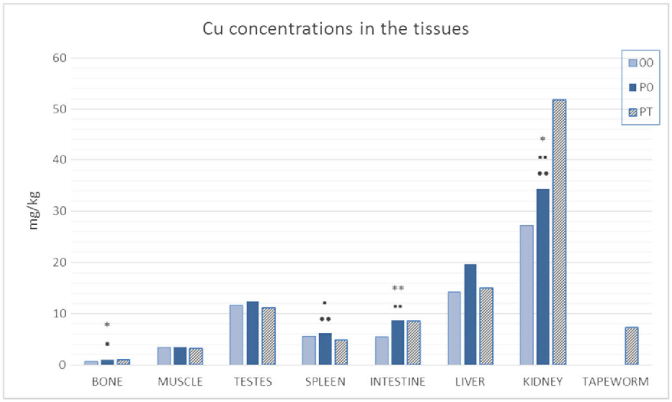
Taking into consideration that cestodes are more abundant in terrestrial mammals than are acanthocephalans (and thus potentially more useful in passive as well as active biomonitoring), we opted for a common animal rat and its common tapeworm (*H. diminuta*) to be used in the present study.

In our experiment, tapeworms decreased only Fe concentration in host tissues (Fig. 2). With regard to apparently lower in infected animals; conversely, Cu concentrations in the kidney tissues were higher in rats with tapeworm infection (Fig. 1). Rat feed mixture (ST-1) contained 20 mg/kg of Cu. Rats from control group (00) were fed 25 g of ST-1/day. Thus, rats from group 00 received 0.5 mg of Cu from the feed mixture (25 g ST-1/day). These rats received a total of 18 mg of Cu over a 6-week period (0.5 mg × 6 days = 3 mg; 3 mg × 6 weeks = 18 mg). The seventh day served as a fasting day (water only).

Tapeworm infection and *A. halleri* (Cd/Zn hyperaccumulating plant) increased Cu concentrations in the kidneys (Fig. 1). Cu concentrations were significantly higher in all tissues for groups PT and P0 (significant increases in kidney, bone, and intestinal tissues) even though Cu levels were higher in the diet provided to control group 00. Whittaker et al. (2011) reported that exposure to Cd and As increased Fe and Cu levels in the kidneys. Copper excess in the kidneys is toxic and can result in various organ dysfunction; copper maldistribution is associated with human genetic diseases such as Menkes disease or Wilson disease (Heo et al. 2010). Increases in Cu concentrations in tissues of rats fed a Cd/Zn hyperaccumulator plant can be caused by the higher amount of Cd. Cadmium can cause a build-up of copper in tissues, which can lead to Wilson's disease. Heo et al. (2010) reported that cadmium salts inhibited the expression of genes related to copper metabolism.

Leitenmaier et al. (2011) isolated and purified a Cd/Zn transporting ATPase of the P_{1B} type from roots of the Cd/Zn hyperaccumulator plant *Noccaea caerulescens*. Mutations in these ATPases can lead to Menkes and Wilson's diseases in humans, which affect the body's ability to maintain the fine balance between copper deficiency and copper toxicity (Bull and Cox 1994).

Rats with tapeworm infection (PT) had significantly lower Fe concentrations in their body tissues than did rats without tapeworms (00, P0, Fig. 2). Iron is a crucial element for both



* statistically significant difference between 00 a P0 groups, p < 0,05; ** statistically significant difference between 00 a P0 groups, p < 0,01
▪ statistically significant difference between 00 a PT groups, p < 0,05; ▪▪ statistically significant difference between 00 a PT groups, p < 0,01
▪ statistically significant difference between P0 a PT groups, p < 0,05; ▪▪ statistically significant difference between P0 a PT groups, p < 0,01

Fig. 1 A comparison of Cu concentrations (mg kg^{-1}) in the tissues of control rats (00) with rats fed a hyperaccumulating plant (*Arabidopsis halleri*) infected (PT) or uninfected (P0) with the tapeworm (*Hymenolepis diminuta*). *statistically significant difference between 00 a P0 groups, $p < 0.05$; **statistically significant difference between 00 a P0 groups, $p < 0.01$ ▪statistically significant difference between 00 a PT groups, $p < 0.05$; ▪▪statistically significant difference between 00 a PT groups, $p < 0.01$ ▪statistically significant difference between P0 a PT groups, $p < 0.05$; ▪▪statistically significant difference between P0 a PT groups, $p < 0.01$

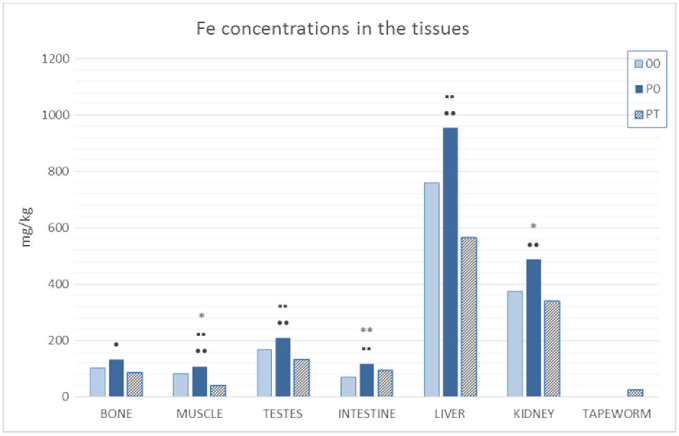


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Fig. 2 A comparison of Fe concentrations (mg kg^{-1}) in the tissues of control rats (00) with rats fed a hyperaccumulating plant (*Arabidopsis halleri*) infected (PT) or uninfected (P0) with the tapeworm (*Hymenolepis diminuta*). *statistically significant difference between 00 a P0 groups, $p < 0.05$; **statistically significant difference between 00 a P0 groups, $p < 0.01$ •statistically significant difference between 00 a PT groups, $p < 0.05$; ••statistically significant difference between 00 a PT groups, $p < 0.01$ •statistically significant difference between P0 a PT groups, $p < 0.05$; ••statistically significant difference between P0 a PT groups, $p < 0.01$

the pathogen/parasite and host in the context of a number of infectious diseases (Navarrete-Perea et al. 2016; Barnet et al. 2007). In vertebrate hosts, parasites can obtain iron from different host sources including erythrocytes, serum hemoglobin, haptoglobin-hemoglobin complexes, hemopexin, transferrin, and lactoferrin (Cassat and Skaar 2013).

In Table 3, Cu, Fe, and Mn concentrations accumulated by the host are compared with those in tapeworm tissues using the following equation: bioconcentration factor BF = tapeworm concentration/host tissue concentration (Sures 2002). Cd and Zn values were described in a previously published work (Jankovská et al. 2016).

The BFs were highest in the muscle tissues for Mn and Fe; however, the BF for Cu was highest in bone tissue. Mn and Zn concentrations were 24.5 and 5.5 times higher, respectively, in tapeworms than in host muscle (Table 3). With regard to Cu, the BF was highest (8) for bone tissues. Cd in the muscle tissues exhibited the highest BF (1551.6).

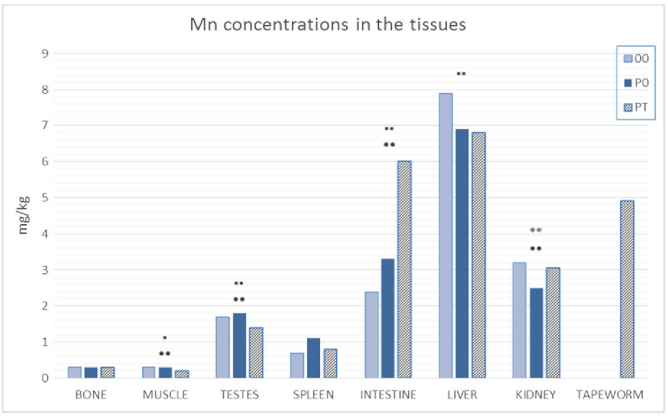
In Fig. 2, we can also see significantly higher Fe concentrations in rats from group P0 (rat fed with Cd/Zn hyperaccumulator plant) than in rats from control group (00). Heo et al. (2010) reported that cadmium exerts negative effects on both iron and copper homeostasis.

Arabidopsis halleri (Cd/Zn hyperaccumulating plant) increased Fe concentrations (Fig. 2) and decreased Mn concentrations (Fig. 3) in the rat livers. Freeland-Graves et al. (2014) also reported that Fe deficiency can be caused by incremental increases in Mn absorption. Manganese concentrations were lower only in muscle and testicular tissues of rats with tapeworm infection, whereas Mn concentrations in the intestinal tissues were higher in rats with tapeworm infection (Fig. 3).

Table 3 Concentrations of copper (Cu), iron (Fe), and manganese (Mn) (mg kg^{-1}) in the tissues of rats of three experimental groups (P0, PT, 00) and bioconcentration factors (BF)

		Cu	BF	Fe	BF	Mn	BF
Kidney	P0	34.3		488.7		2.5	
	PT	51.8	0.1	338.8	0.1	3.1	1.6
	00	27.3		373.8		3.2	
Liver	P0	19.6		953.9		6.9	
	PT	15.0	0.5	564.1	0.043	6.8	0.7
	00	14.2		757.9		7.9	
Bone	P0	1.0		132.8		0.3	
	PT	0.9	8.0	83.8	0.3	0.3	16.3
	00	0.7		102.7		0.3	
Muscle	P0	3.5		106.6		0.3	
	PT	3.2	2.3	38.2	0.6	0.2	24.5
	00	3.4		80.6		0.3	
Testes	P0	12.4		209.9		1.8	
	PT	11.1	0.6	129.9	0.2	1.4	3.5
	00	11.6		166.7		1.7	
Spleen	P0	6.2		5665.5		1.1	
	PT	4.8	1.5	6392.7	0.0003	0.8	6.1
	00	5.6		5702.1		0.7	
Intestine	P0	8.7		117.5		3.3	
	PT	8.5	0.8	92.7	0.3	6.0	0.8
	00	5.4		69.0		2.4	
Tapeworm	PT	7.2		24.0		4.9	

P0—uninfected rats fed the mixture with hyperaccumulating plant *Arabidopsis halleri*; PT—rats with tapeworm infection fed the mixture with the hyperaccumulating plant *Arabidopsis halleri*; 00—control group of uninfected rats fed the standard feed mixture; BF—bioconcentration factor = tapeworm concentration/host tissue concentration



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• statistically significant difference between P0 a PT groups, p < 0,05; •• statistically significant difference between P0 a PT groups, p < 0,01

Fig. 3 A comparison of Mn concentrations (mg kg^{-1}) in the tissues of control rats (00) with rats fed a hyperaccumulating plant (*Arabidopsis halleri*) infected (PT) or uninfected (P0) with the tapeworm (*Hymenolepis diminuta*). *statistically significant difference between 00 a P0 groups, $p < 0.05$; **statistically significant difference between 00 a P0 groups, $p < 0.01$ •statistically significant difference between 00 a PT groups, $p < 0.05$; ••statistically significant difference between 00 a PT groups, $p < 0.01$ •statistically significant difference between P0 a PT groups, $p < 0.05$; ••statistically significant difference between P0 a PT groups, $p < 0.01$

This element is an essential dietary nutrient and trace element, and it plays an important role in mammalian development, metabolism, and antioxidant defense at low concentrations; however, it becomes neurotoxic at higher concentrations (Chua and Morgan 1996; Mercandante et al. 2016). In our experiment, Mn concentrations were the highest in the livers of rats from all groups 00, P0, and PT, with levels of 7.9, 6.9, and 6.8 mg kg^{-1}, respectively (Fig. 3). Jankovská et al. (2009) observed a greater Mn content in the livers of small terrestrial rodents—field voles (*Microtus agrestis*) and bank voles (*Clethrionomys glareolus*)—infected by the tapeworms *Paranocephala* spp. than in those of nonparasitized animals. Regardless of a bioaccumulation of Cu and Mn in the parasites, a significant increase in concentrations of Mn and Cu was observed in the liver of foxes predominantly infected by *Mesocestoides* spp. tapeworms (Jankovská et al. 2010c). In the testes and muscle tissues, Mn concentrations were lower in rats with tapeworm infection (this may support the theory regarding metal absorption by tapeworms from a host body); however, Mn concentrations in the small intestine were significantly higher in rats with tapeworm infection. The third highest Mn concentrations (4.9 mg/kg), after those in the tissues of the liver (6.8 mg/kg) and small intestine (6.0 mg/kg), were observed in the tapeworm (Fig. 3).

The effects of tapeworm presence on the bioaccumulation of heavy metals in the tissues of a host can also serve as an important tool in monitoring environmental pollution.

High concentrations of zinc, iron, and/or calcium in animal feed can reduce the absorption rate of cadmium from various food sources. If dietary zinc is minimal in the diet, cadmium excretion is markedly delayed (Reeves and Chaney 2004). When experimental animals were fed diets containing marginal concentrations of Zn, Fe, and/or Ca, the rates of absorption and whole-body retention of dietary Cd increased seven to tenfold (Reeves and Chaney 2001).

Dietary Zn deficiency resulted in a decrease in Fe levels and an increase in manganese (Mn) and Cu concentrations in the liver, as well as an increase in Fe and a decrease in Cu and Ca levels in the kidneys of Japanese quails fed low doses of Cd (62 μg/kg). This is in contrast to the quails fed a Zn-adequate diet (Fox et al. 1984).

In our study, *A. halleri* (Cd/Zn hyperaccumulating plant) in rat feed (group P0) increased Fe concentrations in the rat livers and kidneys (and also other tissues) (Fig. 2). Contrarily, Mn concentrations were significantly lower in the livers and kidneys of rats fed *A. halleri* in comparison to those of the control animals (Fig. 3).

Zinc in *A. halleri* is bound mainly to malate or other organic acids (Sarret et al. 2009); Cd is also bound to organic acids, cell wall components, and, to a lesser extent, thiol-containing molecules (Huguet et al. 2012). Previously published papers have demonstrated that metals found in plant sources tend to be more easily absorbed than those that come in inorganic forms, which are artificially added to animal feed (Čadkováa et al. 2013). Our recent study (Válek et al. 2015) was the first to use *A. halleri* in a feeding study. Using *A. halleri* in feed stresses the consumer organism due to its Cd content, rather than to its Zn content. Cadmium is primarily acquired through food consumption or inhalation (Lebedová et al. 2016). Intestinal absorption of Cd is proportional to its concentration in the diet; however, other factors also influence the rate of the intestinal absorption and organ retention of Cd. The interaction between Cd and mineral nutrients that are antagonistic to Cd absorption (Reeves and Chaney 2008) poses a significant problem.

There is a lack of scientific literature concerning the behavior of the rat tapeworm *H. diminuta* in the presence of Cd, Cu,

Fe, Mn, and Zn in a host. Sures et al. (2002) carried out the study to observe the effects of this tapeworm on laboratory rats exposed to certain heavy metals. However, interactions between elements and heavy metals (ingested at high doses) still need further studies.

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Compliance with ethical standards All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic Act No. 246/1992 coll. on the Protection of Animals Against Cruelty and EC Directive 86/609/EEC.

Conflict of interest The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

How the tapeworm *Hymenolepis diminuta* affects zinc and cadmium accumulation in a host fed a hyperaccumulating plant (*Arabidopsis halleri*)

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Abstract The effects of plant-bound zinc (Zn) and cadmium (Cd) on element uptake and their interactions in a parasite-host system were investigated in a model experiment. Male Wistar rats were divided into four groups (C, P, TC and TP). Groups TC and TP were infected with the rat tapeworm *Hymenolepis diminuta*. Groups C and TC were fed a standard rodent mixture (ST-1) and received 10.5 mg of Zn per week, while groups P and TP were fed a mixture supplemented with the Zn- and Cd-hyperaccumulating plant *Arabidopsis halleri* at a dosage of 236 mg Zn/week and 3.0 mg Cd/week. Rats were euthanized after 6 weeks, and Cd and Zn levels were determined in rat and tapeworm tissue. The results indicate that tapeworm presence did have an effect on Cd and Zn concentrations in the host tissue; the majority of tissues in infected rats had statistically significant lower Zn and Cd concentrations than did uninfected rats. Tapeworms accumulated more zinc and cadmium than did the majority of host tissues. This important finding confirms the ability of tapeworms to accumulate certain elements (heavy metals) from the host body to their own body tissues. Thus, tapeworms can decrease heavy metal concentrations in host tissues.

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Introduction

Risk element contamination of the environment is a global problem (Brozova et al. 2015; Jankovska et al. 2014; Oprsal et al. 2015; Vaculik et al. 2015; Zarubova et al. 2015).

However, zinc, copper, chromium, manganese and cobalt are also essential trace elements. These are defined as elements contained in low concentrations (mg/kg or less) in plants and animals (Phipps 1981). They are essential for metabolic processes in animals, but at higher levels, they may be toxic. Zinc ensures proper development and an effective immune response. Zinc is the central atom of a wide range of metalloenzymes, and as a part of the insulin molecule, it interferes with the metabolism of sugars (Brown et al., 2001; Brody, 1998; Cuajungco and Lees, 1997; Frederickson et al., 2005; Sun et al. 2011). When zinc is taken at high doses for the long-term, it is absorbed at the expense of other metals, and symptoms of anaemia may develop (WHO 1996; FAO 2001; Hotz and Brown 2004).

Cadmium is a toxic element that is primarily acquired through food consumption. Intestinal absorption of Cd is proportional to its concentration in the diet; however, other factors also influence the rate of the intestinal absorption and organ retention of Cd. One significant problem is the interaction between Cd and other mineral nutrients that are antagonistic to Cd absorption (Reeves and Chaney 2008).

Biogenic (Cu, Cr, Co, Mn, Zn) and toxic (Pb, Cd, Hg) elements in soil can be released (under suitable physicochemical conditions) and become potentially available for plants in areas with elevated levels of these elements. Thus, soil-plant transfer is a possible way for these metals

to become part of the food chain (Cadkova et al. 2013). Certain plants, including *Arabidopsis halleri,* can tolerate or even accumulate very high concentrations of cadmium and zinc. Such plants are known as hyperaccumulators and can considerably accelerate the introduction of soil-bound toxic elements into the food chain. Moreover, these species can also be used in phytoremediation and the monitoring of heavy metals in the environment (Zhenli et al., 2005). The majority of elements found in animals enter through the oral route and are subsequently absorbed in the digestive tract. This absorption process significantly interferes with gastrointestinal parasites, especially acanthocephalans (and tapeworms), which receive nutrients through the tegument, a metabolically active body surface (Sures et al., 2000a, Sures et al., 2000b, Sures et al., 2002; Eira et al. 2005; Kosik-Bogacka et al., 2010). Tapeworms are able to accumulate a considerable amount of metals and reduce their concentrations in host tissues (Jankovska et al. 2011; Cadkova et al. 2013).

The aim of the present study was to investigate the ability of the rat tapeworm *Hymenolepis diminuta* to not only accumulate cadmium and zinc derived from the hyperaccumulating plant *A. halleri* but also affect their concentrations in the tissues of a definitive host (*Rattus norvegicus*).

Material and methods

Breeding and infection of rats

Twenty-four male Wistar rats (*R. norvegicus* var. *alba*) each weighing 150 g, were immediately checked for the presence of the intestinal helminths through a faecal sampling examination.

Rats were divided into four groups of six individuals and kept at a temperature of 21 ± 2 °C and a relative humidity of 75 ± 5 % for 3 weeks. This 3-week period is required for rats to acclimatize and for tapeworms to fully develop in the infected rats. During the acclimatization period (3 weeks), rats were given ad libitum access to both water and a standard ST-1 rodent feed, commercially available from Velaz Ltd. (Table 1).

Infection of rats was initiated with cysticercoids acquired from laboratory-bred beetles (*Tribolium confusum*), which were infected by ingesting tapeworm eggs collected from the excrements of previously infected rats. Cysticercoid development in beetles took place over a 20-day period. The cysticercoids were then collected, suspended in a solution of glucose and administered to the rats orally via micropipettes. Each rat was infected with three cysticercoids.

Table 1 Composition of ST-1

Moisture	%	12.5
Nitrogen compounds	%	24
Fibre	%	4.4
Lipids	%	3.4
Ash	%	6.8
Lysin	mg/kg	14,000
Methionin	mg/kg	4800
Ca	mg/kg	11,000
P	mg/kg	7200
Na	mg/kg	1800
Cu	mg/kg	20
Zn	mg/kg	70
Se	mg/kg	0.38

Experimental design

When tapeworm infection was verified, the rats were housed individually in metabolic cages with a controlled temperature of 21 ± 2 °C and relative humidity 75 ± 5 %. Mode light was set at a 12 h/12 h dark/light cycle. Rat group distribution is shown in Table 2. Over a period of 6 weeks, groups P and TP were given ST-1 (25 g/day) supplemented with dried and homogenized *A. halleri* containing 50.4 mg/kg of Cd and 3912 mg/kg of Zn. Therefore, both groups P and TP received weekly zinc and cadmium doses of 236 and 3.0 mg, respectively. Groups C and TC received only finely minced ST-1, which provided only 10.5 mg of zinc per week (we fed rats only 6 days a week; the seventh day was a fasting day, when rats were provided with water only). Animal body weight was monitored weekly. The EU Legislation limits Zn content in complete feed mixtures to 250 mg/kg (EU regulation 2316/98), i.e., 37.5 mg/kg/week.

Sampling and analytical determination of metals

Six weeks into the study, the rats were euthanized and tissues were taken from the following seven organs with Teflon tools: the liver, small intestine, kidneys, spleen, muscle, testes and

Table 2 Experiment design

Group	*n* of animals	Zn/week (mg)	Cd/week (mg)	*H. diminuta*
Control (C)	6	10.5	–	–
Control + tapeworm (TC)	6	10.5	–	+
Exposure (P)	6	236	3.0	–
Exposure + tapeworm (TP)	6	236	3.0	+

bone tissue (marrow and osseous tissues). Furthermore, tapeworms were removed from the small intestines of the infected rats. All samples were immediately placed in a freezer at −20 °C and subsequently freeze-dried. The samples were then pulverized, and aliquots taking approximately from 400 to 500 mg were decomposed through microwave-assisted digestion using a mixture of 65 % HNO_3 (8.0 ml) and 30 % H_2O_2 (2.0 ml) purchased from Analytica Ltd., Prague, Czech Republic by using the device Ethos 1 (MLS GmbH, Leutkirch, Germany) at 220 °C for 45 min. The digests were poured into 20-ml glass tubes and diluted to 20 ml with distilled water. Certified reference material BCR 185R bovine liver was added to the samples for quality assurance analysis.

Element contents in the digests were determined by inductively coupled plasma-atomic emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA) equipped with a two-channel peristaltic pump, a Struman-Masters spray chamber and a V-groove pneumatic nebulizer made of inert material. To determine low Cd concentrations in the digests, we implemented electrothermal atomic absorption spectrometry (ETAAS) through the use of a VARIAN AA280Z (Varian, Australia) equipped with a GTA120 graphite tube atomizer.

Statistical analysis

Element concentrations and their statistical differences were compared between groups using the nonparametric Mann-Whitney *U* test. All computations were done using Statistica 10 software (Statsoft, USA).

Results and discussion

Our results indicated statistically significant differences between groups of rats infected with tapeworms and their non-infected counterparts (Figs. 1, 2, 3 and 4). The rat group affected by both the Cd- and Zn-hyperaccumulating plant and tapeworms (TP) had significantly lower ($p < 0.01$) concentrations of cadmium and zinc in the majority of their tissues when compared to the non-infected rat group (Figs. 2 and 4).

It was determined in the 1990s that several helminths (primarily acanthocephalants in fish) are able to accumulate considerable concentrations of heavy metals (Lafferty 1997; Sures et al. 1998; Sures 2001; Sures and Siddall 2001; Sures 2003; Thielen et al. 2004). Information regarding whether parasites in terrestrial vertebrates can serve as sentinels for heavy metal environmental pollution, as well as the benefits they provide to their hosts, remains inconsistent (Sures et al. 2002; Baruš et al. 2003; Torres et al. 2004, 2006; Jankovská et al., 2008, Jankovska et al., 2009, Jankovská et al., 2010). Since cestodes are more abundant in terrestrial mammals than are acanthocephalans and, thus, potentially more useful in passive as well as active biomonitoring; a very common animal (*R. norvegicus*) and its common tapeworm (*H. diminuta*) were selected for the present study. As Sures et al. (2002) reported in their lead biomonitoring study, this host-parasite model can be used both as a bioindicator to monitor environmental pollution (especially in urban areas) and as a means to reduce heavy metals in the organs and tissues. As Sures et al. (2002) did with their study dealing with lead concentrations, we compared Cd and Zn concentrations accumulated by the host and those in tapeworm tissues (bioconcentration factor BF = C (tapeworm)/C (host tissue).

With respect to Zn concentrations, tapeworms accumulated 160.34 (TC) and 200.26 (TP) mg kg^{-1}. This translates to 1.9, 2.1, 2.0, 1.1, 2.2 and 4.0 times more Zn than that accumulated in the liver, spleen, kidneys, bone, small intestine and muscles, respectively, of the host from the TC group. Only testis tissue accumulated slightly more zinc than the tapeworms did (Table 3). This can be

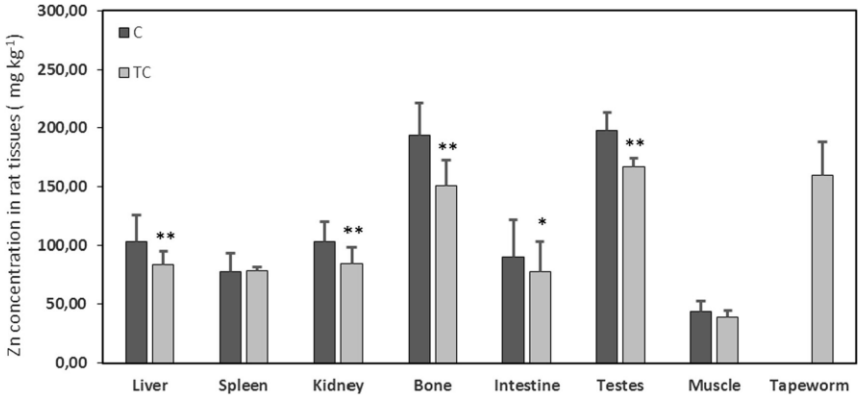

Fig. 1 Zinc concentrations in the tissues of rats fed a standard mixture ST-1 (*C*) and of rats infected by tapeworms (*TC*) $*p \leq 0.05$ $**p \leq 0.01$

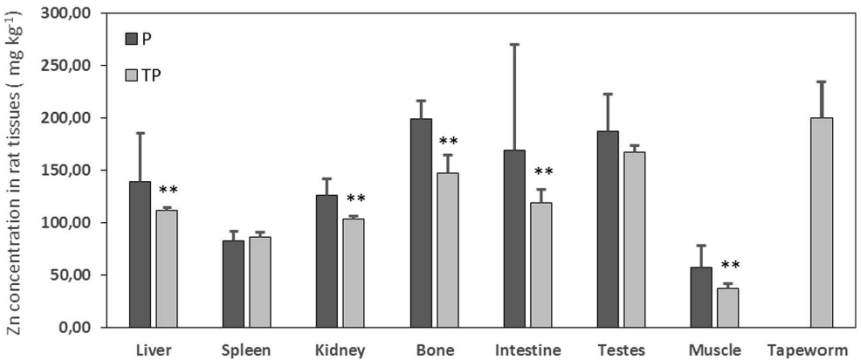

Fig. 2 Zinc concentrations in the tissues of rats fed *Arabidopsis halleri* (*P*) and of rats infected by tapeworms (*TP*) $^{**}p \leq 0.01$

attributed to the protective effects of Zn against testicular damage caused by Cd (Bonda et al., 2004).

It is known that feeding high concentrations of zinc, iron and/or calcium to animals reduces the rate of absorption of cadmium from various food sources. When zinc is marginal in the diet, the delay of cadmium excretion is more pronounced (Reeves and Chaney, 2004). The rates of absorption and whole-body retention of dietary Cd increased 7- to 10-fold when experimental animals were fed diets containing marginal concentrations of Zn, Fe and/or Ca (Reeves and Chaney, 2001, 2002).

In *A. halleri* leaves, Zn is bound mainly to malate or other organic acids (Sarret et al. 2009); Cd is also bound to organic acids, cell wall components and, to a lesser extent, thiol-containing molecules (Huguet et al. 2012). Previously published papers have indicated that metals in plants are more easily absorbed than those in inorganic forms, which are artificially added to animal feed (Cadkova et al. 2013). To our knowledge, Válek et al. (2015) were the first to use *A. halleri* in a feeding study. Recent studies have suggested that using *A. halleri* in feed stresses the consumer organism due to its Cd content, rather than its Zn content. Cadmium (Cd) is an environmental pollutant that is ranked eighth among the top 20 most hazardous substances (Klaassen et al. 2009), and human activity has markedly increased its distribution in the global environment. Zinc is an essential element for all organisms. However, it is toxic when taken in excess (Johnson et al. 2007).

In Tables 3 and 4, we compared zinc and cadmium concentrations between organs of rats with or without parasites and with or without *A. halleri* diet supplementation. Cd concentrations were significantly higher in rats given *Arabidopsis* in their feed mixture (group P); this group (P) had Cd levels that were 329, 147, 87, 39, 10 and 3 times higher in the kidneys, liver, small intestine, testes, spleen and muscle, respectively, than in those of rats not given *Arabidopsis* (group C). Cadmium concentration differences between groups C and TC, as well as between P and TP, are presented in Table 4. There were only slight zinc concentration differences between

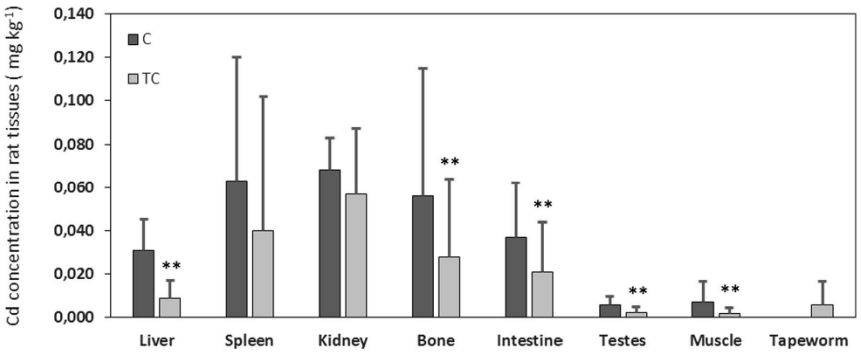

Fig. 3 Cadmium concentrations in the tissues of rats fed a standard mixture ST-1 (*C*) and of rats infected by tapeworms (*TC*) $^{**}p \leq 0.01$

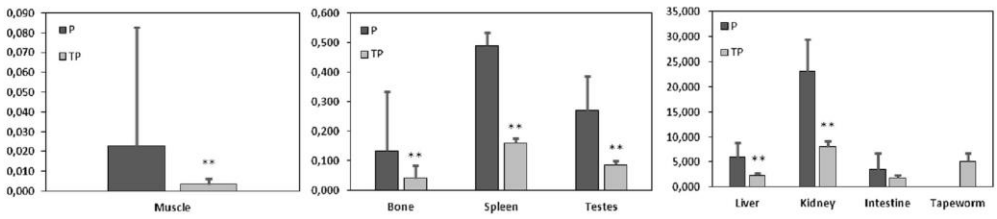

Fig. 4 Cadmium concentrations in the tissues of rats fed *Arabidopsis halleri* (*P*) and of rats infected by tapeworms (*TP*) $^{**}p \leq 0.01$

groups C and TC, P and TP, as well as between P and C, ranging from ratios of 0.9 (testes) to 1.7 (small intestine tissue) as presented in Table 3.

The main site of zinc absorption in animals is the small intestine, where the distal duodenum and proximal jejunum play a key role. Zinc excretion is primarily through faeces (1–5 mg of Zn can be excreted by humans over a 24-h period). Zn levels are directly influenced by the content of zinc in the diet. Stools contain unabsorbed zinc from food, endogenous zinc secreted into the intestine from the pancreas and gallbladder and zinc from the intestinal epithelial cells (Krebs, 2000).

The liver, kidneys, bones and testes of group TC had significantly lower Zn concentrations ($p < 0.01$) than did those of group C (Fig. 1); statistically significant differences between these two groups were also found in the small intestine ($p < 0.05$).

The liver, bones, small intestine, testes and muscles of rats with tapeworms (TC) had significantly lower Cd concentrations (Fig. 3) than did those of group C (non-infected rats).

The liver, kidneys, muscles, bones, testes and spleen of rats infected with tapeworms and fed *A. halleri* (TP) had significantly ($p < 0.01$) lower Cd concentrations (Fig. 4) than did those of the non-infected rats (P). In the TP group, tapeworms accumulated Zn concentrations that were 1.8, 2.3, 1.9, 1.4, 1.7, 1.2 and 5.5 times higher than those accumulated by the liver, spleen, kidneys, bones, small intestine, testes and muscles, respectively (Table 3). Zn concentrations were lower in all host tissues than in the tapeworms. This supports the theory regarding the ability of tapeworms to accumulate heavy metals from the host.

Scheef et al. (2000) described the ability of the acanthocephalan parasite (*Moniliformis moniliformis*) to accumulate cadmium from its rat host (*R. novergicus*). The study lasted 3 weeks, and the rats were exposed to a solution of $CdCl_2$. They found that the parasite accumulated significantly more of this element than did rat tissues. However, there was no indication that cadmium levels in the tissues of infected rats were significantly lower than those in non-infectected rats. Similar results were published by Sures et al. (2000b) in the case of lead, another risk element. They investigated the acanthocephalan parasite *M. moniliformis,* which parasitizes in rats, and found that it accumulated Pb from the host body. They determined that acanthocephalan females contained 25, 39, 2 and 9 times more Pb than did the host liver, small intestine, kidney cortex and kidney medulla, respectively. The ratio of acanthocephalan males was different (7; 11; 0.5 and 3). However, tapeworms are hermaphrodites, so our study could not provide such comparisons. It is evident from both experiments that acanthocephalans have the ability to accumulate higher concentrations of metals than do the host tissues.

In the case of cadmium, tapeworms accumulated 2.2 and 2.6 times higher levels than did the host testis and muscle tissue, respectively (group TC). The remaining host tissues contained higher Cd concentrations than did

Table 3 Zinc concentrations in rat tissues (mg kg^{-1}) and bioconcentration factors (BF)

	Liver	Spleen	Kidney	Bone	Intestine	Testes	Muscle	Tapeworm
C	110.65	77.01	111.08	190.22	102.20	205.33	44.89	
TC	86.08	76.97	82.38	150.86	72.83	167.32	39.79	160.34
BF	*1.9*	*2.1*	*2.0*	*1.1*	*2.2*	*0.96*	*4.0*	
C/TC	*1.3*	*1*	*1.4*	*1.3*	*1.4*	*1.2*	*1.1*	
P	139.34	82.92	126.52	199.45	168.96	186.82	57.24	
TP	111.65	85.82	103.16	146.75	119.26	167.55	36.68	200.26
BF	*1.8*	*2.3*	*1.9*	*1.4*	*1.7*	*1.2*	*5.5*	
P/TP	*1.3*	*1*	*1.2*	*1.4*	*1.4*	*1.1*	*1.6*	
P/C	*1.3*	*1.1*	*1.1*	*1.1*	*1.7*	*0.9*	*1.3*	

BF (bioconcentration factor = concentration in tapeworm/concentration in host tissue) is the concentration accumulated by the host and those in tapeworm tissues; *C/TC, P/TP, P/C* is the share (ratio) of individual groups (C, P, TC, TP)

Table 4 Cadmium concentrations in rat tissues (mg·kg^{-1}) and bioconcentration factors (BF)

	Liver	Spleen	Kidney	Bone	Intestine	Testes	Muscle	Tapeworm
C	0.04	0.047	0.07	0.10	0.04	0.007	0.009	
TC	0.01	0.05	0.05	0.02	0.01	0.003	0.003	0.006
BF	*0.6*	*0.1*	*0.1*	*0.3*	*0. 5*	*2.2*	*2.6*	
C/TC	*4*	*0.9*	*1.4*	*5*	*4*	*2.3*	*3*	
P	5.87	0.49	23.01	0.13	3.46	0.27	0.023	
TP	2.36	0.16	7.94	0.04	1.86	0.09	0.003	5.09
BF	*2.2*	*32.0*	*0.6*	*127.6*	*2.7*	*59.6*	*1551.6*	
P/TP	*2.5*	*3.1*	*2.9*	*3.3*	*1.9*	*3*	*7.7*	
P/C	*147*	*10*	*329*	*1.3*	*87*	*39*	*3*	

BF (bioconcentration factor = concentration in tapeworm/concentration in host tissue) is the concentration accumulated by the host and those in tapeworm tissues; *C/TC, P/TP, P/C* is the share (ratio) of individual groups (C, P, TC, TP)

the tapeworms (Table 4); however, there were only trace quantities in this case since rats from the TC group were not affected by Cd in food.

Group TP rats were affected with cadmium through diet, and the tapeworm Cd concentrations for this group were 2.2, 32.0, 127.6, 2.7, 59.6 and 1551.6 times higher than those in the liver, spleen, bone, small intestine, testes and muscles of host, respectively (Table 4). The kidneys are major Cd-accumulating organs in mammals. This was confirmed by our study; Cd concentrations in kidneys reached 7.94 mg kg^{-1}, which was 1.6 times higher than those in tapeworms (Table 4).

There is not sufficient scientific literature concerning the behaviour of the rat tapeworm *H. diminuta* in the presence of cadmium or zinc in a host. Sures et al. (2002) studied the effects of rat tapeworms (*H. diminuta*) on laboratory rats exposed to lead as $Pb(CH_3COO)_2$. After calculating the bioconcentration factor, they found lead concentrations in the tapeworms that were 17 times higher than those found in the rat kidneys.

Our study determined Zn concentrations in tapeworms that were 1.9 times higher than those in the host kidneys (Table 3, group TP). Contrarily, Cd concentrations in the kidneys of hosts from the same group (TP) were 2.85 mg kg^{-1} higher than those in the tapeworms (Table 4).

Nevertheless, our results showed that tapeworms have a significant effect on zinc and cadmium accumulation in host (rat) tissues. Even though Zn concentrations were similar in both groups (with or without *Arabidopsis*), Cd concentrations were significantly higher in rats given *Arabidopsis* in their feed mixture (group P); this group (P) exhibited Cd levels that were 329, 147, 87, 39, 10 and 3 times higher in the kidneys, liver, small intestine, testes, spleen and muscle, respectively, than in those of rats not given *Arabidopsis* in their feed mixture (group C). Tapeworms accumulated more zinc and cadmium than did the majority of host tissues. For example, tapeworms accumulated 5.5 times more Zn and 1542 times more Cd than did the host muscle tissue. Moreover, when we compared group TC (standard feed mixture and tapeworm infection) with group TP (feed mixture with added hyperaccumulating plants and tapeworm infection), we found that tapeworms from group TP accumulated 848 times more Cd than did tapeworms from group TC.

Since few comparative studies on heavy metal concentrations in tissues of infected and uninfected hosts are available, it remains unclear if conspicuous metal accumulation by parasitic worms affects metal levels in the tissues of the definitive host.

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Compliance with ethical standards All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic Act No. 246/1992 coll. on the Protection of Animals against Cruelty and EC Directive 86/609/EEC.

Conflict of interest The authors declare that they have no conflict of interest.

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EFFECTS OF TWO CADMIUM HYPERACCUMULATING PLANTS (*N. CAERULESCENS* AND *A. HALLERI*) IN FEED ON TISSUE BURDEN IN LABORATORY RATS*

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The aim of this work was to determine how two cadmium (Cd) hyperaccumulating plants in feed affect a consumer organism (*Rattus norvegicus* var. *alba*). Using inductively coupled plasma optical emission spectrometry (ICP-OES), Cd concentrations were analyzed in Wistar rat (*Rattus norvegicus* var. *alba*) tissues. Rats were fed the Cd and Zn hyperaccumulating plants *Noccaea caerulescens* or *Arabidopsis halleri*. Rats given *Arabidopsis halleri* took in 4 times as much Cd as did rats fed *Noccaea caerulescens*. However, the muscle, intestinal, kidney, spleen, testicular, bone and liver tissues of rats fed *A.halleri* had 7.3, 5.6, 5.5, 3.5, 3.1, 2.5 and 2.3 times higher Cd concentrations, respectively, than did tissues of rats fed *N. caerulescens*. *A. halleri* burdened the muscle, small intestinal, and kidney tissues with Cd to a greater extent than did *N. caerulescens*. However, the spleen, testes, bone and liver were significantly more burdened with Cd by *N. caerulescens*. In both experimental groups (rats given *N. caerulescens* as well as those given *A. halleri*), the highest Cd concentrations were found (in descending order) in the kidneys > liver > small intestine > spleen > testes > bone > and muscle. This information is vital in situations where, for example, livestock can graze on these plants or when other animals and humans accidentally consume these plants.

Arabidopsis halleri, *Noccaea caerulescens*, toxic, cadmium, accumulation

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INTRODUCTION

Contamination of the environment with heavy metals and other elements has become a problem not only in the Czech Republic (Jankovska et al., 2016; Sloup et al., 2016, 2017; Vymazal, 2017) but also in many other surrounding regions (Malaspina et al., 2014; Chen et al., 2016; Chatterjee et al., 2017). Cadmium (Cd) is an environmental pollutant ranked eighth in the Top 20 Hazardous Substances Priority List (Klaassen et al., 2009), and human activity has markedly increased the distribution of Cd in the global environment. One of the primary means of contamination for humans is through the diet, with most foods potentially containing natural or synthetic chemicals that could represent a toxic hazard to the consumer (Raad et al., 2014).

Plant species that colonize polluted environments are tolerant to pollutats or have developed defence mechanisms. Certain plants are able to hyperaccumulate metal ions that are toxic to other organisms at low doses. This trait could be utilized in the cleanup of metal-contaminated soils. Moreover, the accumulation of heavy metals by plants affects both

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the micronutrient content and toxic metal content of our food (Clemens, 2006). Phytoextraction refers to the uptake of contaminants from soil or water by plant roots and their translocation to any harvestable plant part. Phytoextraction has the potential to remove contaminants and promote long-term cleanup of soil or wastewater.

Noccaea caerulescens syn. *Thlaspi caerulescens* (Brassicaceae) is a Cd/Zn hyperaccumulating plant species. It has attracted the interest of plant biologists due to its ability to colonize calamine and serpentine soils, which contain naturally elevated levels of heavy metals such as Zn, Pb, Cd, Ni, Cr and Co (Pence et al., 2000).

Arabidopsis halleri syn. *Cardaminopsis halleri* (Brassicaceae) serves as a model plant for Zn and Cd hyperaccumulation. It is Zn-tolerant and a Zn-hyperaccumulator (Meyer et al., 2010). *A. halleri* is also moderately tolerant to Cd (Zhao et al., 2006). Huguet et al. (2012) determined that the mechanisms of Cd storage and detoxification in *A. halleri* differ from those that were previously revealed for Zn. Hyperaccumulators are important tools for the phytoremediation of Cd-contaminated soil (Liu et al. 2011).

The capability to hyperaccumulate heavy metals in *A. halleri* and *N. caerulescens* is achieved by duplications and alterations of the *cis*-regulatory properties of genes coding for heavy metal transporting/excreting proteins (Bothe, Slomka, 2017).

In a previously published study (Valek et al., 2015), we monitored the effects of *A. halleri* on rats; in the current study we compared two hyperaccumulating plants and their effects on a consumer organism (rats). The aim of this work was to compare how two hyperaccumulating plants (*Arabidopsis halleri* and *Noccaea caerulescens*) can affect Cd concentrations in the individual tissues of a consumer organism (*Rattus norvegicus* var. *alba*).

MATERIAL AND METHODS

Experimental design

Arabidopsis halleri and *Noccaea caerulescens* aboveground biomass was sampled in the flowering stage under natural conditions of an area contaminated with Cd and Zn in the vicinity of Příbram (Czech Republic). These plant samples were later dried at laboratory temperature and homogenized.

Experimental animals

The present experiment was conducted on 12 male Wistar rats over a six-week period. The rats were randomly divided into two experimental groups (*Arabidopsis halleri* group and *Noccaea caerulescens* group). Rats from the experimental groups (*Arabidopsis halleri* group and *Noccaea caerulescens* group) were fed a mixture of ST-1 (60%) and dried and homogenized plants (40%). Rats given the *A. halleri* plant took in 4 times as much Cd as did rats fed the *N. caerulescens* plant (33.5 mg vs 7.8 mg Cd in 6 weeks).

Animal welfare

During the experiment, all animals were placed in individual cages. The room housing the cages was air-conditioned. A constant temperature (22–24°C) and humidity level (approximately 70%) were maintained during a constant day/night cycle (8:00–20:00 h). The animals were provided free access to water. All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic (Act No. 246/1992 coll. on the Protection of Animals against Cruelty).

Sampling and analytical procedure

Six weeks into the study, the rats were euthanized and tissues were taken from the following 7 organs with Teflon tools: the liver, small intestine, kidneys, spleen, muscle, testes, and bone tissue (marrow and osseous tissues). All samples were immediately placed in a freezer at –20°C and subsequently freeze-dried. The samples were then pulverized, and 400–500 mg aliquots were decomposed through microwave assisted digestion using a mixture of 65% HNO_3 (8.0 ml) and 30% H_2O_2 (2.0 ml), purchased from Analytica Ltd. (Prague, Czech Republic) using an Ethos 1 (MLS GmbH, Leutkirch, Germany), at 220°C for 45 min. The digests were poured into 20 ml glass tubes and diluted to 20 ml with distilled water. Certified reference material BCR 185R bovine liver was added to the samples for quality assurance analysis.

Element content in the digests was determined using inductively coupled plasma-atomic emission spectrometry (ICP-OES) (Agilent 720; Agilent Technologies Inc., USA) equipped with a two channel peristaltic pump, a Struman-Masters spray chamber, and a V-groove pneumatic nebulizer made of inert material. To detect low Cd concentrations in the digests, we implemented electrothermal atomic absorption spectrometry (ETAAS) through the use of a VARIAN AA280Z (Varian, Australia) equipped with a GTA120 graphite tube atomizer.

Statistical analysis

Cd 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 STATISTICA version 10 program (Statsoft, USA).

RESULTS

Cd accumulation in rat tissues (bone, small intestine, kidney, liver, spleen, testis, muscle) after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*) is shown in Figs. 1–7. Rats fed *N. caerulescens* as well as those fed *A. halleri* had the highest Cd concentrations in the following tissues in descending order: kidney > liver > intestinal > spleen > testis > bone > muscle tissues (Figs. 1–7). Total Cd intake by rats given *N. caerulescens* over a six-week period was 7.8 mg Cd per kg. The mean Cd concentrations in rat tissues were as follows (in $mg.kg^{-1}$): 0.003 in the muscle, 0.06 in the bone, 0.07 in the testes, 0.15 in the spleen, 0.50 in the small intestine, 2.22 in the liver, and 4.46 in the kidneys (Figs. 1–7). The sum total of Cd levels in the investigated organ tissues was 7.463 mg Cd per kg, which accounts for 96% of the Cd taken up by *N. caerulescens*.

Total Cd intake from *A. halleri* over a six-week period totalled 6.03 mg Cd per rat (180 g), which corresponds to 33.5 mg Cd per kg. Cadmium concentrations in rat tissues were as follows (in $mg.kg^{-1}$): 0.02 in the muscle, 0.15 in the bone, 0.22 in the testes, 0.52 in the spleen, 2.80 in the small intestine, 5.19 in the liver, and 24.58 in the kidneys. The sum total of Cd in the investigated organ tissues was 33.462 mg Cd per kg, which accounts for 99.9% of the Cd taken up by *A. halleri*.

A. halleri burdened the muscle, small intestinal, and kidney tissues with Cd to a greater extent than did *N. caerulescens*. However, the spleen, testes, bone and liver were significantly more burdened with Cd by *N. caerulescens*.

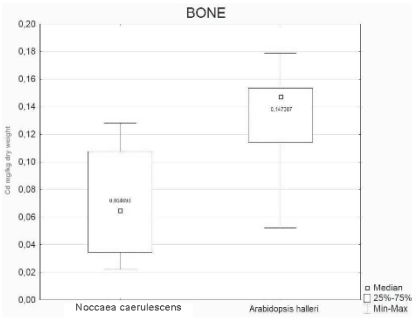

Fig. 1. Cd accumulation in the rat bone after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.02 ($P < 0.05$)

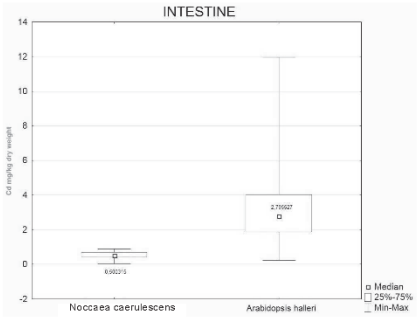

Fig. 2. Cd accumulation in the rat small intestines after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.002 ($P < 0.01$)

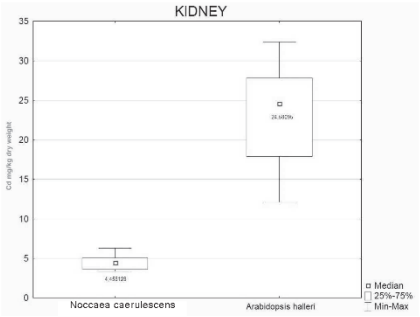

Fig. 3. Cd accumulation in the rat kidney after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.000055 ($P < 0.01$

DISCUSSION

Toxic metal ions that enter plant roots pose a potential threat to human health (McLaughlin et al., 1999). Cadmium is of particular concern because it is among metals whose ions are most readily taken up by plant roots (Wagner, 1993). *A. halleri* is widely distributed throughout Europe, and it is present in contaminated and non-contaminated areas (Bert et al., 2002).

The ability of *A. halleri* to accumulate and tolerate Cd is comparable to that of the well-known Cd hyperaccumulator *N. caerulescens*. Kupper et al. (2000) reported that *A.halleri* is able to accumulate up to 6000 $mg.kg^{-1}$ of Cd on a dry-weight (DW) basis in the shoots; however, phytotoxicity was observed at this level. Although *A. halleri* can accumulate up to 6000 $mg.kg^{-1}$ DW of Cd when grown in hydroponic solution, plants in their natural European habitats do not normally accumulate more than 100 $mg.kg^{-1}$ DW (Huguet et al., 2012).

In our study, *A. halleri* and *N. caerulescens* grew in the natural conditions of an area contaminated with

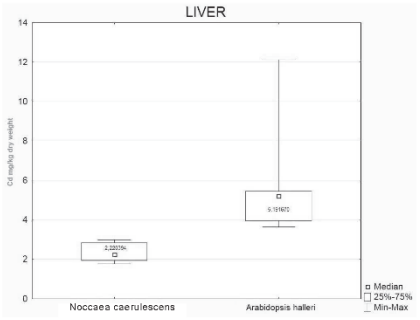

Fig. 4. Cd accumulation in the rat liver after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.000037 ($P < 0.01$)

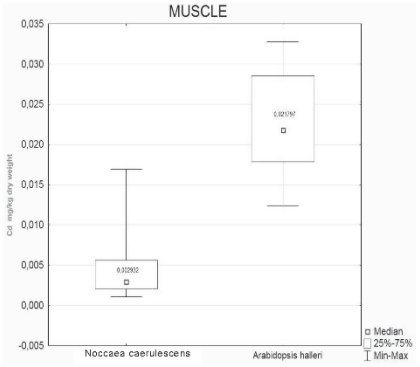

Fig. 7. Cd accumulation in the rat muscle after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.00008 ($P < 0.01$)

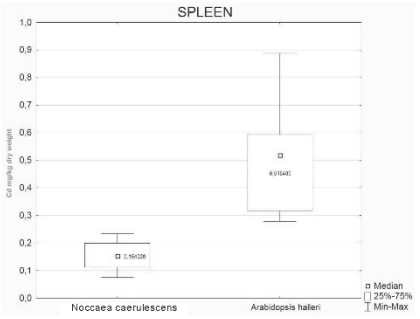

Fig. 5. Cd accumulation in the rat spleen after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.00014 ($P < 0.01$)

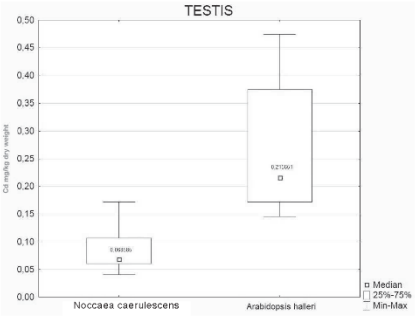

Fig. 6. Cd accumulation in the rat testis after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.00008 ($P < 0.01$)

Cd and Zn near the city of Příbram, Czech Republic. *A. halleri* grew naturally at this locality, whereas *N. caerulescens* was seeded there.

Although rats given *A.halleri* in feed mixture took in 4 times as much (33.5 mg) Cd over a six-week period as did rats fed *N. caerulescens* (7.8 mg Cd per 6 weeks), kidney, intestinal and muscle tissues of rats given *A.halleri* contained 5.5, 5.6 and 7.3 times higher Cd concentrations, respectively, than those tissues of rats fed with *N. caerulescens*. Contrarily, the liver, bone, testis and spleen tissues of rats fed *A. halleri* contained only 2.3, 2.5, 3.1, and 3.5 times as much Cd, respectively, as did those of rats fed *Noccaea caerulescens* (Figs. 1–7).

These results suggest that *A. halleri* affects the consumer organism through the accumualtion of high levels of Cd, especially in the kidney, intestinal and muscle tissues.

CONCLUSION

In this study, we revealed that *A. halleri* (Cd/Zn hyperaccumulating plant) affected the consumer organism (*Rattus norwegicus* var. *alba*) with cadmium significantly more than other Cd/Zn hyperaccumulating plant (*N. caerulescens*). There has been little literature (Valek et al., 2015) to date that deals with the effects of hyperaccumulating plants on consumer organisms. This study is arguably the first to shed light on these problems. However, further research will be required to fully clarify these problems.

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

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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

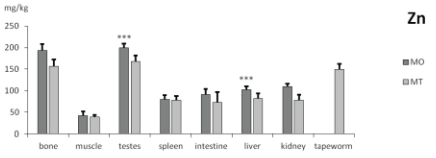

Fig.1a. Zinc concentrations (mg kg–1) in rat tissues and rat tapeworm M0 = rats with zinc lactate in feed mixture, MT = rats with zinc

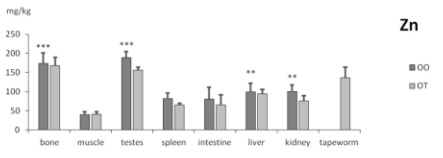

Fig. 1b. Zinc concentrations (mg kg–1) in rat tissues and rat tapeworm 00 = control rats, 0T = control rats with tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

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 metic, 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

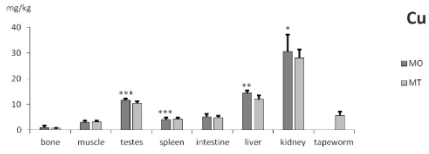

Fig. 2a. Copper concentrations (mg kg 1) in rat tissues and rat tapeworm M0 = rats with zinc lactate in feed mixture, MT = rats with zinc lactate in feed mixture and tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

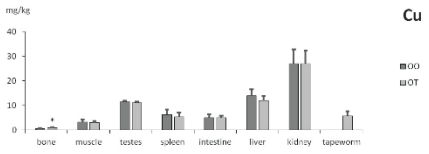

Fig. 2b. Copper concentrations (mg kg 1) in rat tissues and rat tapeworm 00 = control rats, 0T = control rats with tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

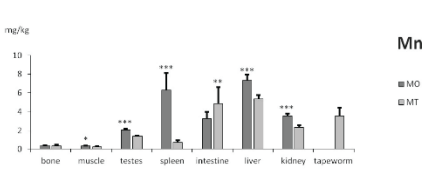

Fig. 3a. Manganese concentrations (mg kg–1) in rat tissues and rat tapeworm M0 = rats with zinc lactate in feed mixture, MT = rats with zinc lactate in feed mixture and tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

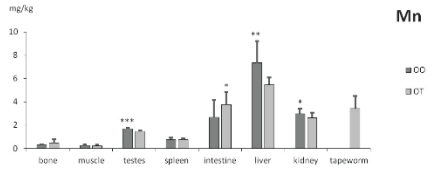

Fig. 3b. Manganese concentrations (mg kg–1) in rat tissues and rat tapeworm 00 = control rats, 0T = control rats with tapeworm infection statistically significant difference between groups ***P > 0.001, **P ≥ 0.05, *P ≥ 0.01

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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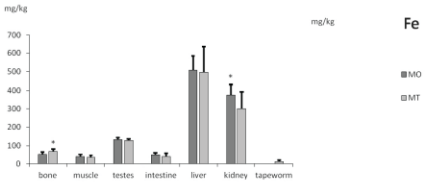

Fig. 4a. Iron concentrations (mg kg–1) in rat tissues and rat tapeworm M0 = rats with zinc lactate in feed mixture, MT = rats with zinc lactate in feed mixture and tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

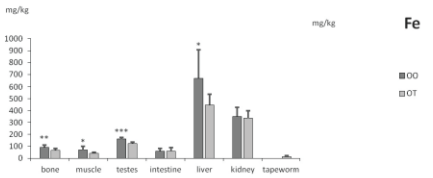

Fig. 4b. Iron concentrations (mg kg–1) in rat tissues and rat tapeworm 00 = control rats, 0T = control rats with tapeworm infection statistically significant difference between groups ***P ≥ 0.001, **P ≥ 0.05, *P ≥ 0.01

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ZINC IN THE ANIMAL ORGANISM: A REVIEW*

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Zinc, as an essential metal, is necessary for the correct function of an organism. It is involved in biochemical processes that affect the immune response of an organism, and it acts as a neuromodulator in the excitatory synapses of the brain. Zinc is also applied in response to stressful stimuli. Zinc is an essential factor of gene expression and is important, at the cellular level, in maintaining the integrity of the cell walls. It influences organism ageing. Zinc is relatively abundant in nature, and it exists in a mineral form and rarely as a pure element. Zinc is used widely in industry and agriculture. In industry, it is utilized mainly in the processing of other metals as protection against corrosion. In agriculture, it is used in fertilizers and chemicals to produce pesticides. In certain areas affected by human activities, its concentrations increase, and large quantities of this metal can get into the food supply. In this paper, we focus on zinc metabolism and homeostasis, with an emphasis placed on the biological function of zinc. This study also deals with zinc deficiency and its effect on health. We also touch on the excessive intake of zinc and its toxicity.

metal, enzyme, protein, metallothionein

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INTRODUCTION

Zinc (Zn) is one of the most important nutrients for animal health. Numerous proteins, crucial enzymes, and transcription factors bind to Zn and are thought to be dependent on Zn for their functions. Zn is involved in many biochemical processes that support life. The most important of these are cellular respiration, cellular use of oxygen, DNA and RNA expression, maintenance of cell membrane integrity, sequestration of free radicals, and protection against lipid peroxidation. Zn is a trace element that is a central component of metalloenzymes, lactate dehydrogenase, carboxypeptidase, and DNA and RNA polymerases. The human body contains 1.5–2.5 g of Zn, with 60% found in muscle and 30% in bones (Fig. 1). The recommended dose of zinc is 11 mg/day for adult men and 8 mg/day for adult women (Cousins, 1998; Brown et al., 2001; Erdman et al., 2012 – see Table 1).

Zn is an essential metal involved in many biochemical processes, and it is associated with a wide range of physiological defects, including disorders of the skin, anorexia, weight loss, growth retardation, and impaired neurologic and immune systems. Zn deficiency in children depresses growth, appetite, skeletal maturation, and gonad development, which can be reversed with Zn treatment. Zn deficiency also causes alterations in the activities of some enzymes such as ALP, copper/Zn superoxide dismutase (Cu-Zn SOD), carboxypeptidases, DNA and RNA polymerases, and lactate dehydrogenase (Cuajungco, Lees, 1997; Brody 1998; Frederickson et al. 2005; Sun et al., 2011).

Zn is involved in the maintenance of gut structure and function, and plays a vital role in gut immune function. Zn deficiency causes villi of the jejunum to become shrivelled and flattened. This change in morphology decreases surface area absorption, and there

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was a substantial decrease in the number of villi per unit area. Zn supplementation leads to an accelerated regeneration of the mucosa and to increased levels of brush border enzymes (Sun et al., 2011).

Nutritional Zn deficiency is a worldwide problem (Fig. 2). The risk of inadequate Zn intake through diet is greatest in Africa, the Middle East, and South America. Insufficient intake of Zn is associated with protein-energy malnutrition (Wessells, Brown, 2012). Zn deficiency is associated with metabolic disturbances in a wide range of hormones, cytokines, and enzymes involved in growth and bone development (e.g. insulin-like growing factor-I (IGF-1), growth hormone, thyroid hormone, insulin, prolactin, alkaline phosphatase (ALP), and prostaglandins). Inadequate Zn intake in both humans and animals has been shown to cause growth retardation and delayed skeletal maturation. ALP, a Zn-metalloenzyme found on the surface of osteoblasts, is essential for normal bone formation and/or mineralization. Bone-specific ALP contains two Zn molecules per enzyme monomer, and this enzyme-bound Zn is required for ALP activity. Moreover, removal of Zn by chelation results in an irreversible loss of bone-specific ALP activity. Previous animal studies indicated that bone-specific ALP activity decreased in the bones of Zn-deficient rats. In addition, there is a dose-dependent relationship between dietary Zn and skeletal ALP in the tibia of adult female mice. Carbonic anhydrase II (CAII), a Zn-containing enzyme that catalyzes the reversible hydration of carbon dioxide, is an initial regulator of osteoblast differentiation, and is essential in optimal bone resorption (Kukačka et al., 2008; Sun et al., 2011).

Proteins for Zn transport, zinc finger proteins, and metallothioneins (MTs) are all non-enzymatic zinc proteins. Two protein families have been implicated in Zn transport: ZnT proteins and Zip proteins. Dysregulation in Zn transport via specific protein transporters has been linked to specific diseases. MTs, a superfamily of non-enzymatic peptides with low molecular mass and a unique sequence of amino acids, play a significant biological role in immunoregulation, neuroprotection, metalloregulation, and detoxification (Ladomery, Dellaire, 2002; Kukačka et al., 2008).

An overdose of Zn is not possible through a normal diet. In the cases where nutritional supplements enriched by Zn are taken in high doses, the metabolism of other metals may be disturbed; dietary Zn has anantagonistic effect on Cu absorption. Animals given low amounts of Zn retained more Cu than did animals fed high levels of Zn (Fisher et al., 1981). When Zn is taken in extremely high doses, it is absorbed in the intestines at the expense of other metal ions, and the amount of Cu, Fe, Co or Cr decreases. If this continues unchanged, symptoms of anaemia can arise. This means we must ensure a balanced intake of all biogenic metals in order to prevent any distortion of stability during penetration of the intestinal wall (Ferguson et al., 1995; Brody, 1998; Brown et al., 2001).

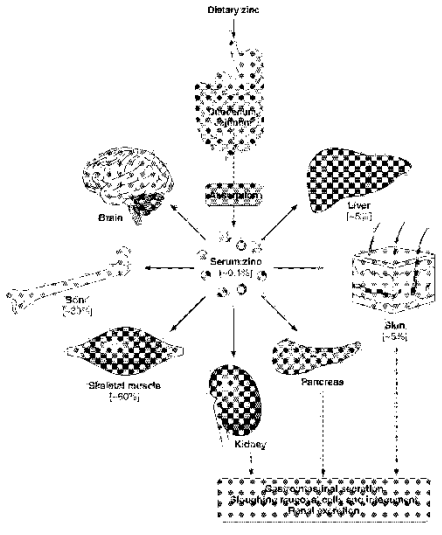

Fig. 1. The distribution of zinc in various tissues (Kambe et al., 2015)

Zinc contamination of agroecosystems

Zinc, like other trace elements (Cu, Mn, Co, Ni), enters an agroecosystem in two ways. The first way is during the weathering of parent material that contains high concentrations of minerals containing Zn. The three main minerals that contain Zn are smithsonite ($ZnCO_3$), sphalerite (ZnS), and hemimorphite ($Zn_4Si_2O_7(OH)_2 \cdot H_2O$). Basalts, for example, are igneous rocks with high levels of Zn. Sedimentary rocks with high levels of Zn are known as slates (Zhenli et al., 2005). These minerals may rapidly weather under the influence of specific environmental conditions. Trace elements in them are oxidized and thus become mobile (Shuman, 1991; Zhenli et al., 2005).

The second method of entry is through anthropogenic activity. Anthropogenic processes include the application of fertilizers, fungicides, herbicides, pesticides, and pond sediments on agricultural land, which contains levels of various trace elements including Zn. For example, the application of agricultural chemicals in orchards may increase Zn content by 5–9 kg/ha of land per year (Shuman, 1991; Zhenli et al., 2005). Zn also enters agro-ecosystems via animal excreta. Zn is an inorganic antimicrobial compound, and in order to prevent excessive Zn in agricultural

Table 1. The recommended daily intake of zinc by age and sex (Erdman et al., 2012)

Life stage	Age	AI (mg/day)		EAR (mg/day)		RDA (mg/day)		UL (mg/day)	
		Male	Female	Male	Female	Male	Female	Male	Female
Infants	0-6 months	2.0	2.0					4.0	4.0
	7-12 months			2.5	2.5	3.0	3.0	5.0	5.0
Children	1-3 years			2.5	2.5	3.0	3.0	7.0	7.0
	4-8 years			4.0	4.0	5.0	5.0	12.0	12.0
	9-13 years			7.0	7.0	8.0	8.0	23.0	23.0
	14-18 years			9.5	7.3	11.0	9.0	34.0	34.0
Adults	= 19 years			9.4	6.8	11.0	8.0	40.0	40.0
Pregnancy	14-18 years				10.0		12.0		34.0
	19-50 years				9.5		11.0		40.0
Lactation	14-18 years				10.9		13.0		34.0
	19-50 years				10.4		12.0		40.0

AI, adequate intake; EAR, estimated average requirement; RDA, recommended dietary allowance; UL, tolerable upper intake level.

land, EU legislation has limited Zn content in complete feed mixtures to 250 mg/kg or 80 mg/kg in chelated Zn (Comission Regulation (EC) No. 2316/98). Only small portions of trace elements in soil are bioavailable. Trace element mobility and accessibility are affected by many chemicals and biochemical processes such as dissolving in rainwater, adsorption–desorption, complexation reactions, dissociation, and oxidation-reduction reactions. Zn mobility is affected by soil pH as well as various biological processes (Zhenli et al., 2005).

Heavy metal pollution is a consequence of human activity that affects all components of an ecosystem, mainly the soil. Many authors have analyzed how plants respond to this pollution and plant ability to accumulate metal in different tissues (Berchová-Bímová et al., 2014; Bfendová et al., 2015; Száková et al., 2016; Tlustoš et al., 2016).

Zinc metabolism

Food is the main source of Zn. Relatively high concentrations of this element are found in meat (especially beef and pork, but also turkey and chicken), seafood (oysters are considered a great Zn source), cereals, and legumes. Generally, animal-based foods are a more preferable source of Zn than plant-based foods. Animal-based foods contain hardly any compounds that inhibit Zn absorption. Especially zero phytate content is the important thing. Phytic acid reduces bioavailability of Zn by forming insoluble complexes together. The presence of certain amino

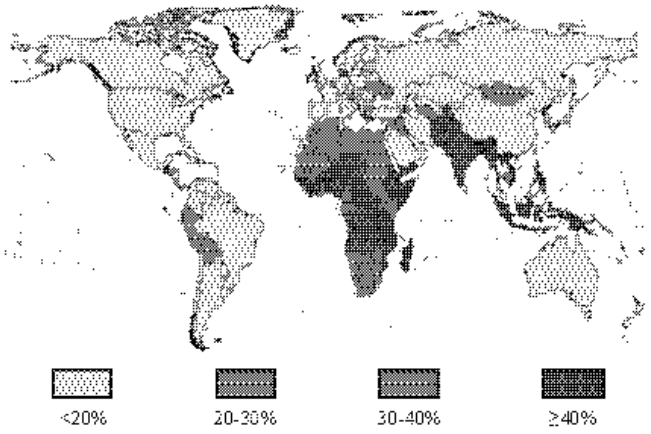

Fig. 3. Estimate of inadequate intake of zinc from food in individual countries (Wessels, Brown, 2012)

acids improves absorption of Zn, cysteine, and histidine (Brown et al., 2001).

Zinc absorption

The main site of Zn absorption in animals is the small intestine, where the distal duodenum and proximal jejunum play a key role. Zn absorption takes place by two means: active and passive transport. Active transport is carried out by specific transporters, and its effectiveness increases with increasing dietary Zn intake. Passive transport operates on a diffusion mechanism, and its effectiveness is proportional to the concentration of Zn in the intestinal lumen (Cousins, 1998; Krebs, 2000).

Zn absorption at the cellular level is a process of entry into the enterocyte and Zn through the basolateral membrane transports into the portal circulation. This process is carried out using several proteins known as zinc transporters (Cousins, 1998; Krebs, 2000).

Zinc distribution

Absorbed Zn is transported in the plasma bound mostly to albumin (60–80%), less on a-2-macroglobulin and transferrin, but also bound to free amino acids, especially histidine and cysteine. Zn bound to plasma proteins is the most freely available and easily available supply of Zn in the body, although it represents only about 0.1% of the total amount of Zn in the body. Blood contains five times more Zn than plasma. In erythrocytes, 80% of Zn is contained in carbonic anhydrase and Zn-superoxide dismutase (Cousins et al., 2006).

Zn transported to the liver is later released into the body. In hepatocytes, enterocytes, and other cells, Zn is kept in custody on metalloproteins. Metalloproteins include metalloenzymes, gene regulation molecules, storage proteins, and zinc transporters. Zn in hepatocytes is primarily bound to MTs (Cousins et al., 2006).

Zinc transporters

Zinc transporters are divided into two groups: ZnT and ZIP. Forwarders group ZnT exports Zn out of the cytoplasm. ZnTs are found mainly on the cytoplasmic membrane, Golgi, endosome, and the endoplasmic reticulum. This group includes ZnT-1, which is located on the plasma membrane. This protein transports Zn from the cell into the extracellular space in almost all tissues. Furthermore, ZnT-2 also transports Zn out of the cell, but it also has the ability to transport Zn into storage vesicles in the cell (under conditions of high concentrations of Zn in the cell). This function is carried out mainly in the acinar cells of the pancreas. Furthermore, it is located in the intestine, kidneys, and testes. The ZnT-3 activity is associated with the transfer of Zn to vesicles, and its expression is restricted to the brain. This demonstrates the important role of Zn in the central nervous system. ZnT-4 is located in the mammalian glands and brain. ZnT-5 is localized on the vesicles secretory cells of the pancreas and the apical membrane of the enterocytes (apical part of the plasma membrane is for absorption; this area is called brush border). ZnT-10 is localized on the cytoplasmic membrane (Robinson et al., 2001; Coyle et al., 2002; Huang et al., 2005; Cousins et al., 2006; Kukačka et al., 2008).

The ZIP group of carriers can be divided into four subgroups: Zip I, Zip II, gufA, and LZT. Most proteins of ZIP group (Zip 4–8, Zip 10, and Zip 12–14) belong to subgroup LZT. Zip 1–3 belong to subgroup ZIP II, Zip 9 belongs to subgroup ZIP I, and protein Zip 11 is a member of subgroup gufA. The zinc transporter Zip 6 of LZT subgroup was subsequently included in a separate subgroup labelled LIV-1 (Robinson et al., 2001; Coyle et al., 2002; Cousins et al., 2006; Kukačka et al., 2008).

It was found that ZIP zinc is transferred into the cells cytoplasm from the extracellular space or intracellular vesicles. Most of them are located on the cytoplasmic membrane. However, Zip 7 is located in the Golgi. ZIP 14 is mobilized to the membrane in the hepatocytes in the case of acute inflammation, thereby increasing Zn absorption (Robinson et al., 2001; Coyle et al., 2002; Kukačka et al., 2008).

Metallothionein

Metallothioneins (MTs) are a group of intracellular proteins with low molecular weight. MT can bind to divalent metal cations, including Zn. MT is composed of 60–68 amino acids, twenty of which are cysteines. The human genome contains at least 16 genes for MT. These genes encode proteins with closely related sequences, and are expressed in different tissue types – mainly in the liver, kidneys, intestine, pancreas, and brain (Robinson et al., 2001; Coyle et al., 2002). All MTs are characterized not only by a high content of cysteine, but also by the binding of metal ions by thiolates and the creation of cysteinyl–thiolate clusters with a characteristic spatial arrangement (Alberts et al., 1998; Miles et al., 2000; Adam et al., 2008).

MT performs many functions in the body, the most important being the transport of essential metal ions and detoxification of toxic levels of metal ions. Most of MTs bind zinc. MTs act also as a reservoir of excess metal ions. They can be mobilized during conditions of insufficient metal ion intake (Robinson et al., 2001; Coyle et al., 2002).

The mammalian MT contains 61 to 68 amino acid residues, in which 18 to 23 cysteine residues are present. None is an aromatic amino acid or histidine. The chain of amino acids is as follows: Cys-Cys, Cys-X-Cys, and Cys-X-X-Cys (X represents an amino acid differ-

ent from cysteine). This is characteristic of MT. The tertiary structure of MTs is composed of two domains (α: stable domain containing the C-terminal end, and β: reactive domain containing the N-terminal end). They coordinate 7 divalent or 12 monovalent metal ions. In organisms, MT occurs in several isoforms: MT-1, MT-2, MT-3, and MT-4 (Quaife et al., 1994; Miles et al., 2000; Vašák, Hasler, 2000; Mejáre, Bűlow, 2001; Coyle et al., 2002).

Regulation of the MT expression is associated with the presence of metal ions. Transcription is initiated after the establishment of metal-regulatory transcription factor-1 (MTF-1) to the metal responsive element (MRE), which lies on the MT gene promoter. Normally, the MTF-1 are bound to MTI, an inhibitor that prevents binding MTF-1 MRE. After entering the metal ion into the intracellular compartment cell creates links between the ion and MTI. Thus the MTF-1 is released and induces the expression of MT (Masters et al., 1994; Adam et al., 2008).

Zinc excretion

Following oral exposure, Zn is primarily excreted via the gastrointestinal tract and eliminated in the faeces; approximately 70–80% of an ingested dose is excreted (Davies, Nightingale, 1975). Pancreatic zinc secretion at homeostasis is 2-4 times the size of the dietary contribution into the duodenum (Oberleas, 1996); most of this secreted zinc is later reabsorbed. It depends primarily on the Zn content in the diet. The amount of Zn found in compound feed for livestock is around 100 mg/kg. Stools contain unabsorbed Zn from food, Zn contained in released intestinal epithelial cells, and endogenous Zn secreted into the intestine from the pancreas and gallbladder (Koyama et al., 1993; Krebs, 2000).

Endogenous intestinal losses can range from 0.5 to 3 mg/day, depending on Zn intake. In normal healthy subjects approximately 0.7 mg Zn per day is lost through urine. Starvation and muscle catabolism increase Zn losses in urine and faeces. The loss of Zn in perspiration and desquamated epidermal cells has been estimated at 0.5 mg/day in adult men; however, this depends on Zn intake (Krebs, 2000).

In humans, approximately 14% of eliminated Zn is excreted in urine; when Zn intake increases, urinary excretion accounts for 25% of eliminated Zn (Wastney et al., 1986). Other minor routes of elimination include sweat (Prasad et al., 1963), saliva secretion (Greger, Sickles, 1979), and incorporation into hair (Rivlin, 1983).

The rate, at which Zn is excreted, is dependent on both current and past Zn intake (Johnson et al., 1988). Age also affects the rate at which Zn is excreted. He et al. (1991) reported that following an intraperitoneal dose of Zn, adult mice had higher levels of Zn in faecal excretions than weanling, adolescent, or young adult mice.

Zinc supplementation in farm animal diets

The reproductive well-being and performance of farm animals is largely dependent on their nutritional status. Micronutrients are especially involved in functions such as intracellular detoxification of free radicals, synthesis of reproductive steroids and other hormones, carbohydrate, protein, and nucleic acid metabolism. Their deficiency and/or excess may impair spermatogenesis and libido in male, fertility, embryonic development and survival, postpartum recovery activities, milk production, and offspring development and survival (Smith, Akinbamijo, 2000). In animals, Zn deficiency can also be manifested through changes in taste perception (accompanied by the tongue epithelium damage), a disorder of keratin synthesis, limited limb bone growth, and sight disorders (Hosnedlová et al., 2007). The mechanism of growth retardation in the case of Zn deficiency can be seen in loss of appetite, imperfect use of nutrients from feedstuffs, and in disorders of the protein and energy metabolism (Illek et al., 2000). One specific disorder resulting from Zn deficiency is parakeratosis – a disorder of the epidermal layer of the skin that occurs in calves, sheep, goats, and piglets. In calves, it is manifested by a characteristic coat shedding that occurs on the head, neck, limbs, and around the eyes ('glasses') (Suchý et al., 1998). Similar findings were observed in free living ruminants by Abdou (2005). Ali et al. (1998) compared two groups of mature ewes that were fed either a control diet containing 23–25 ppm Zn or a test diet supplemented with additional 100 ppm in form of zinc sulphate. Supplementation began one month before mating and continued until lambing period. The above mentioned authors reported that ewes given Zn supplements consumed by about 15% more feed than the controls, had a higher fertility rate, were more prolific (89 vs 40%), and produced heavier lambs at birth (4.0 vs 2.9 kg) and at weaning (17.7 vs14.2 kg).

It has been known for some time that feeding high concentrations of Zn, Fe and/or Ca to animals reduces the rate of absorption of Cd from various food sources. When Zn was marginal in the diet, the delay of Cd excretion was more pronounced (Reeves, Chaney, 2004). The rates of dietary Cd absorption and whole-body retention increased 7–10 fold when experimental animals were fed diets containing marginal concentrations of Zn, Fe and/or Ca (Reeves, Chaney, 2001, 2002).

Excess Zn intake is a relatively rare occurrence in farm animals. It transpires, for example, in piglets treated with medications high in Zn. Excess Zn reduces the digestibility of phosphorus, and causes anaemia and digestive disorders. Poisoning is conditioned

primarily by the antagonistic relationship of Zn to Fe and Cu (Suchý et al., 1998). Noakes et al. (2001) note that excess intake of Zn additives may lead to a disorder of essential fatty acid metabolism, which influences prostaglandin synthesis.

Matrix metalloproteinase

Matrix metalloproteinases (MMPs) are a large group of Zn-dependent proteins that are responsible for cleavage and the adjustment of individual components of connective tissue such as collagen, elastin, gelatin, and casein. Degradation of connective tissue with intensive participation of MMP is a process that takes place during ontogenetic changes in the organism such as growth, morphogenesis, as well as wound healing and tissue damage. It also includes MMP activity in diseases and pathological processes closely related with tissues, e.g. inflammation, tumours, and skin diseases. Most of these enzymes (excluding membrane MMPs) are secreted from the cells in the form of a proenzyme which is activated as needed by Zn ions for actual cleavage rate. Among other things, in the MMP molecule there are Ca ions with structural function. MMPs are homologous proteins, which can be divided into six categories: collagenase, stromelysin, matrilysin, gelatinase, membrane metalloproteinase, and other MMPs. MMPs are Zn- and Ca-dependent endopeptidases, and they are synthesized as inactive preproenzymes (zymogens). This inactive form prevents MMPs from cleaving to the essential components of the cell. MMPs are mostly secreted from cells as inactive proenzymes, except the membrane-bound MMPs (Vanwart, Birkedalhansen, 1990; Masters et al., 1994; Aimes, Quigley, 1995).

Extracellular enzyme activation involves two steps: the first is the initial cleavage of the propeptides of MMP by the protease and destabilization of the propeptide binding interactions. The second is cleavage of the propeptides by other MMPs (Nagase et al., 1991; Aimes, Quigley, 1995; Anand-Apte et al., 1996; Ogata et al., 2001).

Transcriptional function of zinc

There are more than 2000 transcription factors or DNA binding proteins that are dependent on zinc. They are involved in the gene expression of various proteins. These are regulatory proteins that bind to the promoter region in the DNA and allow the initiation of transcription (i.e. the transcription of DNA into RNA). Transcriptional factors binding to DNA have specific structural motifs (the amino acid sequences) to enable this relationship. One of the most important structural motifs is the so-called 'zinc finger'(Ladomery, Dellaire, 2002; Sun et al., 2006).

'Zinc fingers' are protein domains that occur in many different transcription factors, and they allow a (transcription) factor to bind to a specific DNA sequence. The name is inspired by their shape, which consists of a sequence of roughly 30 amino acids strongly bound to the Zn atom. This amino acid sequence contains, among other things, two histidine and two cysteine residues. They are coordinated to one Zn atom. Therefore, 'zinc fingers' usually refer to Cys2His2. The Zn atom is required to bind protein to DNA. Perhaps the most well-known member of this group is the commonly occurring factor Sp1, whose DNA binding domain is composed of three zinc fingers (Ladomery, Dellaire, 2002; Sun et al., 2006).

Proteins that comprise zinc fingers are important in transcription, translation, cytoskeleton organization, developing epithelial cell adhesion, chromatin remodelling proteins, and the arrangement of tertiary structures (Cousins, 1998; Sun et al., 2006).

Another group in which Zn plays an important role in DNA binding are the steroid hormone receptors. These receptors are located in the cytoplasm or nucleus.

Synthesis of insulin

Zinc is indispensable for the synthesis and effectivity of the insulin hormone. Insulin is stored in b-cells of pancreatic islets (in secretory vesicles of Langerhans islets). Into the bloodstream it is carried continuously and its level increases with increasing levels of blood glucose (Prasad, 1998).

Insulin hormones are arranged in a regular crystalline structure comprising Zn ions in secretory vesicles. Each insulin molecule is linked with 2–4 Zn atoms. A zinc/insulin complex is formed for the purpose of slow release of insulin into the bloodstream (Prasad, 1998).

CONCLUSION

Zinc is indispensable for correct growth and development of an organism. It is involved in DNA replication and RNA transcription. In addition to its role in the transcription and translation of genetic material, Zn has an important function in the primary endocrine system, which is involved in growth hormone metabolism of somatotropin. Zinc is associated with reduced concentrations of circulating growth factor, which is similar to insulin-like growth factor 1 (IGF-1), and it is necessary for the correct function of somatotropin. Experiments on animals have shown that Zn deficiency leads to a decrease in food intake, in comparison with control animals administered the correct amount of Zn in feed. In a population that experienced growth retardation, growth was renewed and body weight increased following Zn supplementation

Zinc is extremely important for the immune system. When Zn is deficient, thymic atrophy occurs and thymulin activity is reduced. Thymulin is a hor-

mone that requires Zn in order to become active. Thymulin hormone is responsible for the maturation of T-lymphocytes, cytotoxicity, and cytokine production. Moreover, the development of B-lymphocytes and production of antibodies (especially immunoglobulin G (IgG)) are disturbed. The role of Zn in these immunological processes derives from its involvement in basic cellular functions such as DNA replication, RNA transcription, cell division, and cell activation.

Zinc is also an important antioxidant factor. It is involved in protecting biological structures from free radical damage by maintaining sufficient levels of MT. Zn is an essential component of the enzyme superoxide dismutase (SOD), protects thiol groups (-SH) from oxidation, prevents interaction between the thiol groups, helps prevent lipid peroxidation in mitochondria and microsomal membranes, stabilizes the structure of cell membranes, and prevents damage to the osmotic membranes of erythrocytes. The *p53* tumour suppressor gene (the most frequently mutated gene in human cancer patients) is a transcription factor that contains Zn, and its expression is dependent on this element. This has shown to be an extremely significant finding.

Programmed cell death, or apoptosis, is a regulated biological mechanism that is vital for the health of the immune system. It has been found that Zn plays an important role in the regulation of apoptosis. Zn has the ability to block apoptosis that is induced by external factors.

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ANIMAL SCIENCES

CHANGES OF SOME BIOCHEMICAL PARAMETERS IN RATS SUPPLEMENTED WITH HIGH DOSES OF ZINC LACTATE*

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The experiment was conducted on 18 Wistar rats during a six-week period; 12 animals were given zinc lactate (120 mg/rat and week) in feed mixture and 6 control animals were fed a standard mixture for rats (ST-1). Sixteen biochemical parameters were measured from blood (serum) samples: total protein (TP), albumin (ALB), urea (UREA), glucose (GLU), triacylglycerols (TAG), non-esterified fatty acids (NEFA), cholesterol (CHOL), creatinine (CREAT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), uric acid (UA), magnesium (Mg), calcium (Ca), phosphorus (P), and trace elements such as Fe and Zn. When compared to the control group, we found that rats fed zinc lactate had higher concentrations of GLU, UA, UREA, Fe, Mg, Ca, TAG, TP, ALB, and ALP in the blood serum. Contrarily, the concentrations of AST, NEFA, CHOL, CREAT, P, and Zn were higher in the blood serum of control rats. Statistically significant differences between rats fed Zn and the control were found only in the concentrations of GLU, AST, ALP, UA, and P.

rat, blood, serum, toxicity, glucose

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INTRODUCTION

Zinc (Zn) is an essential element of exceptional biological and public health importance (Hambidge, Krebs, 2007). Zinc plays an important role in a wide variety of metabolic processes in animal systems. However, it is toxic when taken up in excess. Supplementary Zn in the diet increases the probability of zinc toxicity, especially the chronic type (Mizari et al., 2012). There is also strong evidence that depression is associated with low Zn levels in the blood serum (Tao et al., 2013). To prevent the occurrence of Zn deficiency, Zn fortification and supplementation is widely implemented. However, the potential risks of Zn overdosing are largely underestimated (Yang et al., 2013).

Zinc oxide, one of the most common supplements in the United States, and zinc carbonate are nearly insoluble and poorly absorbed in the body. Allen (1998) described low plasma Zn concentrations after consumption of zinc oxide and zinc carbonate if compared to those reached after zinc acetate and sulphate salts supplementation (Allen, 1998).

However, harmful excess supplementation is a problem among the relatively affluent, and dosage should probably not exceed 20 mg/day in healthy people, although the U.S. National Research Council set a tolerable upper intake of 40 mg/day (Maret, Sandstead, 2006).

Approximately 225–450 mg Zn is known to produce immediate vomiting in adults (Fosmire, 1990). Chronic overdosage of Zn, in the range of 100–300 mg Zn/day for adults, may induce copper deficiency (Prasad et al., 1978) and alterations in the immune response and serum lipoprotein levels. Some of these disturbances may also occur at lower doses (50 mg Zn/day) (Plum et al., 2010). 60 mg of supplementary Zn per day resulted in adverse interactions with other nutrients (WHO, 1996). Individuals may be exposed to high Zn intakes, either through supplementation or by contact with environmental zinc. Overt toxicity symptoms, such as nausea, vomiting, epigastric pain,

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diarrhea, lethargy, and fatigue, may occur with acute, high zinc intakes (F o s m i r e , 1990).

Compared to several other metal ions with similar chemical properties, zinc is relatively harmless. Just the exposure to high doses has toxic effects, making acute zinc intoxication a rare event. In addition to acute intoxication, long-term, high-dose Zn supplementation interferes with the uptake of copper.

Zinc homeostasis seems to be an important factor allowing efficient handling of the excess orally ingested zinc (P l u m et al., 2010).

The objective of the present research was to determine possible homeostasis changes of certain biochemical parameters that occur after administering high doses of zinc lactate, which is commonly used by humans as a dietary supplement, using an experimental animal model (laboratory rats *Rattus norvegicus* var. *alba*), and to point to the possible impact of Zn nutritional supplement overdose on the recipient (mammal).

Our hypothesis was that zinc lactate overdosing causes a glucose disbalance in the blood serum, as well as a disbalance in certain biochemical parameters.

MATERIAL AND METHODS

Experimental animals

The experiment was conducted during a six-week period on 18 Wistar rats (initial body weight of animals 150 g). During the experiment each animal was placed in a metabolic cage (1 animal per cage) in an air-conditioned room with constant temperature (22–24°C), humidity level (approximately 70%), and day/night cycle (08:00–20:00 h). The animals had free access to water.

Experimental design

Rats in the control group (Group C, 6 animals) were fed a commercially manufactured feed ST-1 (Table 1), while rats in the experimental group (Group P, 12 animals) were fed a commercial feed mixture ST-1 with zinc lactate (25 g of feed contained 20 mg Zn; each individual was given 150 g feed (6 × 25 g) per week, i.e. 120 mg Zn per week, i.e. 720 mg Zn per 6 weeks).

Biochemical analysis

The blood was collected from *cavum abdominis*. The blood samples were coagulated at laboratory temperature and then centrifuged at 1000 *g* for 15 min. Separated serum was deep-frozen (–80°C) until the analysis. Sixteen biochemical parameters were measured from blood (serum) samples: total protein (TP), albumin (ALB), urea (UREA), glucose (GLU), triacylglycerols (TAG), non-esterified fatty acids (NEFA), cholesterol (CHOL), creatinine (CREAT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), uric acid (UA), minerals such as magnesium (Mg), calcium (Ca), phosphorus (P), along with the concentrations of trace elements (Fe, Zn). Zinc was determined by a manual method using a spectrophotometer (Libra S6; Biochrom, Cambourne, UK). The commercial kit Randox Zinc (Randox Laboratories Ltd., Crumlin, UK) was used for colorimetric method performance. Other blood parameters were determined spectrophotometrically using an automatic analyzer ERBA XL 200 (Erba Diagnostics Mannheim GmbH, Mannheim, Germany) in the laboratory of the Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague. The analysis was performed with commercial kits (namely from Randox NEFA (Randox Laboratories Ltd.) and others kits from Erba Diagnostics Mannheim GmbH.

Table 1. Composition of rat diet (ST-1)

Ingredients	Value
Crude protein	25.5 (%)
Ash	6.26 (%)
Dry matter	86.5 (%)
Crude fibre	4.3 (%)
Nitrogen free extract	47.7 (%)
Fats	3.76 (g/100 g)
Ca	12 700 (mg/kg)
K	10 500 (mg/kg)
Mg	2 210 (mg/kg)
Na	1 780 (mg/kg)
P	8 110 (mg/kg)
Zn	70 (mg/kg)

Statistical analysis

Normality of data was tested separately using a Shapiro-Wilk test, and to evaluate the proposed hypothesis we used non-parametric Mann-Whitney U tests. A P-value of 0.05 was set for our hypothesis about the significant statistical influence of zinc in rat food. STATISTICA 10 (Statsoft, USA) was used for all computations and statistical analysis.

RESULTS

There were no abnormal signs of toxicity or death recorded in Group P after the six weeks of treatment at the dose of 20 mg zinc lactate per rat and day. Gradual body weight increase was comparable with that of control rats, the mean weight gain in Group C was

Table 2. Mean values ± SD of biochemical parameters in blood serum of rats

	Mg (mmol/l)	Ca (mmol/l)	P (mmol/l)*	CHOL (mmol/l)	TAG (mmol/l)*	UREA (mmol/l)	GLU (mmol/l)*	NEFA (mmol/l)
Group P	0.8 ± ·.19	2.5 ± 0.14	2.8 ± 0.48	1.0 ± 0.14	0.8 ± 0.28	6.3 ± 0.66	14.7 ± 2.17	0.6 ± 0.09
Group C	0.7 ± 0.17	2.2 ± 0.33	5.7 ± 2.28	1.5 ± 0.43	0.3 ± 0.12	6.8 ± 0.81	8.3 ± 1.66	0.7 ± 0.32
	ALP (µkat/l)*	AST (µkat/l)*	TP (g/l)	ALB (g/l)	UA (µmol/l)	CREAT (µmol/l)	Fe (µmol/l)	Zn (µmol/l)
Group P	2.7 ± 0.79	1.7 ± 0.24	51.4 ± 2.7	29.3 ± 0.97	144.0 ± 57.15	41.9 ± 4.16	61.1 ± 7.18	23.4 ± 3.45
Group C	1.6 ± 0.42	3.6 ± 1.78	50.3 ± 6.25	28.1 ± 3.44	113.6 ± 85.13	43.5 ± 11.89	54.8 ± 6.79	26.9 ± 6.48

Group P = group treated with zinc lactate, Group C = control group, CHOL = cholesterol, TAG = triacylglycerols, GLU = glucose, NEFA = nonesterified fatty acids, ALP = alkaline phosphatase, AST = aspartate aminotransferase, TP = total protein, ALB = albumin, UA = uric acid, CREAT = creatinine

*statistically significant difference

82 g (mean initial weight 244.5 g and mean final weight 326.5 g); Group P had mean weight gain 61.2 g (mean initial weight 281.4 g and mean final weight 342.6 g). When compared to the control group, we found that rats in Group P showed a higher concentration of GLU, UA, UREA, Fe, Mg, Ca, TAG, TP, ALB, and ALP in blood serum. Contrarily, AST, NEFA, CHOL, CREAT, P, and Zn concentrations were lower in the blood serum of treated rats (Figs. 1–4). Statistically significant differences between groups were in GLU, AST, ALP, UA, and P concentrations in blood serum (Table 2).

DISCUSSION

As shown in Fig. 1, the serum concentrations of Mg, Ca, CHOL, UREA, and NEFA were not significantly affected by treatments with zinc lactate. However, the serum concentrations of GLU, P, and TAG were significantly ($P \leq 0.05$) affected by the addition of zinc lactate to the feed mixture. GLU and TAG concentrations were significantly higher in the blood serum of Group P with Zn supplementation (interestingly, Iciek et al. (2009) and Dvořáková et al. (2015) mentioned low levels of CHOL and TAG after garlic consumption). P concentrations were significantly lower in the blood serum of Group P. Rats in Group C had P concentrations exceeding the upper reference limit of 1.0–3.6 mmol/l (Johnson-Delaney, 1996). Since the rats were only 2 months old and still growing, higher levels of P can be attributed to increased intestinal absorption and decreased renal excretion to facilitate bone mineralization (Gruenberg, 2011).

In our study, glucose concentrations were surprisingly significantly higher in Group P with zinc lactate supplementation, although in some studies (e.g. Uyanik et al., 2001; Baltaci et al. 2003; Dehshal et al., 2007; Bonakdaran et al., 2009) an increased glucose concentration in serum resulted from Zn deficiency, as Zn is associated with insulin synthesis, storage, and secretion. Zinc lactate is commonly used as an easily digestible form of Zn supplement. But lactate as a gluconeogenetic source of glucose may increase serum glucose level (Fig. 1). The glucose level may be also affected by stress (stress hormones adrenaline and cortisol increase gluconeogenesis). Dvořáková et al. (2015) described a decrease in plasma glucose levels after garlic consumption, preventing the development of diabetes mellitus.

The high level of glucose in the serum of rats supplemented with zinc lactate (20 mg/day) may be associated with the lactate form of Zn. Lactate is a part for anaerobic glycolysis. It is the starting compound of

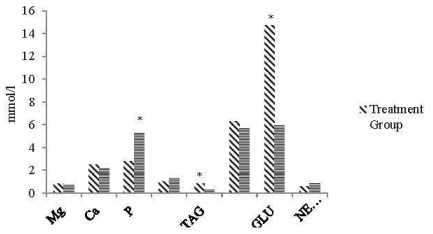

Fig. 1. Effect of oral treatment/intake of zinc lactate (20 mg/rat/day) on biochemical parameters (mmol/l) in rats

CHOL = cholesterol, TAG = triacylglycerol, GLU = glucose, NEFA = nonesterified fatty acids

*statistically significant difference

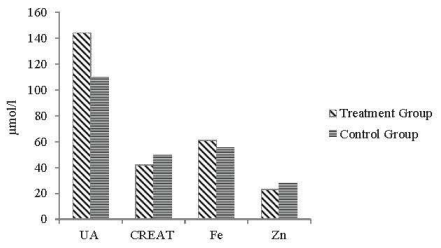

Fig. 2. Effect of oral treatment/intake of zinc lactate (20 mg/rat and day) on biochemical parameters (µmol/l) in rats

UA = uric acid, CREAT = creatinine

gluconeogenesis. Increased amount of zinc lactate in feed mixture promotes the formation of glucose in the liver (Cori cycle). Cori cycle refers to the metabolic pathway in which lactate produced by anaerobic glycolysis in the muscles moves to the liver and is converted to glucose, which then returns to the muscles and is metabolized back to lactate (Nelson, Cox, 2005).

Disorder in mineral status may impact lipid and glucose metabolism, and also mineral dependent enzyme activity, such as that of superoxide dismutase and catalase in the body (Sulibursk a et al., 2014).

Mizari et al. (2012) described significant changes in Na^+, Ca^{2+}, and K^+ concentrations both in saliva and in plasma of rats with oral Zn intoxication. The serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, glucose levels in the plasma, and urine creatinine levels were also altered in experimental groups in comparison with the control group. Furthermore, results showed that zinc toxicity affects the liver and renal function (Mizari et al., 2012).

The primary importance of measuring alkaline phosphatase (ALP) is to check the possibility of bone or liver disease. Since the mucosal cells that line the bile system of the liver are the source of ALP, the free flow of bile through the liver and down into the biliary tract and gallbladder is responsible for maintaining the proper level of this enzyme in the blood. When the liver, bile ducts or gallbladder systems are not functioning properly or are blocked, this enzyme is not excreted through the bile and ALP is released into the blood stream. Higher ALP concentrations in rats from the treatment group can be related to Zn excess. Thus, the serum ALP is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine. Increased ALP is typical for bile ducts disease. The causes of decreased serum ALP may be zinc deficiency, hypothyroidism, vitamin C deficiency/ Scurvy, folic acid deficiency, excess vitamin D intake, low P levels (hypophosphatasia), celiac disease, malnutrition with low protein assimilation (including low stomach acid production/ hypochlorhydria), insufficient parathyroid gland function, pernicious anemia, or vitamin B_6 insufficiency (Kaslow, 2014). ALP activity is inhibited by a low Zn concentration. In our study, the concentration of ALP in blood serum was significantly higher in Group P. It can be the result of higher Zn concentration in rat body.

Aspartate aminotransferase (AST) catalyses the reversible transfer of α-amino group between aspartate and glutamate and, as such, it is an important enzyme in the amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health. The blood test for AST is usually used to detect liver damage (Zhang et al., 2004). In our study, the concentration of AST in rat serum significantly differed between the treatment Group P and the control Group C (Fig. 3). However, AST concentration was higher in Group C compared to Group P rats (Fig. 3). Awe, Banjoko (2013) reported that the activity of AST showed insignificant change in rats supplemented with various doses of *Petrosellinium crispum* ethanol extracts (10, 100, and 1000 mg/kg body weight). However, the activity of plasma ALP increased (78 and 88 IU/l) at doses of 100 and 1000 mg/kg body weight.

In our study, a higher uric acid (UA) concentration in Group P was observed (Fig. 2); the increase of serum UA values is a consequence of renal injury after a high intake of metal-containing substances (Garban et al., 2013). There was no statistically significant difference in blood serum Zn concentrations between Groups P and C (Table 2). Zn concentration was surprisingly a little higher (21.7–40.5 μmol/l) in Group C (rats without zinc lactate supplementation) in comparison to Group P supplemented with zinc lactate (17.8–29.0 μmol/l) (Fig. 2). Zn supplementation did not increase the serum Zn concentration, indicating that Zn levels were not reflected in blood Zn concentrations. However, persistent intake of high doses of Zn can lead to copper deficiency (Rink, Gabriel, 2000).

Protein in the plasma is made up of albumin and globulin. Albumin is mainly produced in the liver. It helps keep the blood from leaking out of blood vessels. Albumin also helps carry some medicines and other substances through blood and is important for tissue growth and healing. In blood plasma, zinc is bound to and transported by albumin (60%) and transferrin (10%) (Rink, Gabriel, 2000). Since transferrin also transports iron, excessive Fe reduces Zn absorption, and *vice versa*. A similar reaction occurs with copper. The concentration of Zn in blood plasma stays relatively constant regardless of the Zn intake (Whitney, Rolfes, 2013)). Zinc lactate in the feed mixture did not significantly influence total protein and albumin concentrations in rat blood serum.

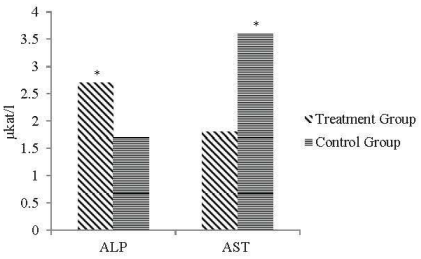

Fig. 3. Effect of oral treatment/intake of zinc lactate (20 mg/rat and day) on biochemical parameters (μkat/l) in rats
ALP = alcalic phosphatase, AST = aspartate aminotransferase
*statistically significant difference

The results of Yang et al. (2015) indicate that Zn plays an important role in hippocampus-dependent learning, memory, and brain-derived neurotrophic factor (BDNF) expression. A high dose supplementation with Zn induces specific Zn deficiency in the hippocampus; this deficiency further impairs learning and memory due to a BDNF deficit and a decreased availability of synaptic Zn. Elevated temperature, infection, stress, or pregnancy decreased the Zn concentration in plasma, while it was increased by fasting and catabolism (decomposition) (King et al., 2000). Interestingly, zinc lactate overdosing did not increase Zn concentrations in blood serum in our study.

Negative effects of some elements, however, arise not only from anthropogenic pollution (Berchová-Bímová et al., 2014; Weingartová et al., 2015), but also from excessive use of nutritional supplements which was confirmed also by the present results.

CONCLUSION

Blood is the main tissue that transports the metabolites and xenobiotics in the organism. The interactions of various metal compounds with biological systems are very important. Zinc lactate is well absorbed by humans and animals, and that is why it is commonly used as a dietary supplement. The present research revealed statistically significant differences between concentrations of GLU, AST, ALP, TAG, and P in the group given a zinc lactate overdose and the control group. Zinc lactate overdosing significantly increased GLU, ALP, and TAG levels, while significantly decreased AST and P. We have come to the conclusion that increased levels of GLU, ALP, TAG and decreased levels of AST and P could indicate a zinc lactate overdose. These findings should be taken into consideration when diagnosing suspected Zn overdose.

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Can the Hyperaccumulating Plant *Arabidopsis halleri* in Feed Influence a Given Consumer Organism (*Rattus norvegicus* var. *alba*)?

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Abstract Zinc and cadmium concentrations in rat (*Rattus norvegicus* var. *alba*) tissues were analyzed by inductively coupled plasma optical emission spectrometry. Rats were fed the zinc and cadmium hyperaccumulating plant, *Arabidopsis halleri*. When compared to the control group, a Cd increase in all tissues (liver, kidneys, small intestine, spleen, testes, muscle), with the exception of bone tissue was observed. In comparison to the control group, the kidneys, liver and small intestine contained 375, 162, and 80 times more Cd, respectively. Differences between zinc concentrations in rats fed with *A. halleri* and those of the control group were significant only in the small intestine and kidney tissues. Results suggest using the hyperaccumulating plant *A. halleri* as a feed stresses the consumer organism not through its Zn content, but through its Cd content.

Keywords Rat · Tissue · Accumulation · Zinc · Cadmium

Metal contamination has become a worldwide problem (Dadar et al. 2014; Jankovská et al. 2010; Magdaleno et al. 2014; Petrović et al. 2013). Cadmium (Cd) is an environmental pollutant ranked eighth among the top 20 hazardous substances priority list (Klaassen et al. 2009), and human activity has markedly increased the distribution of Cd in the global environment. Zinc (Zn) is an essential element for all organisms. However, it is toxic when taken in excess (Johnson et al. 2001).

Metal concentrations in agricultural soils can become elevated due to anthropogenic emissions as well as from geological origins of the soil (Garrett 2000; McLaughlin et al. 1999). Some metals can easily transfer to aerial parts of plants and thus enter the food chain (Meyer et al. 2010; Zhao et al. 2006). Phytoextraction refers to the uptake of contaminants from soil or water by plant roots and their translocation to any harvestable plant part. Phytoextraction has the potential to remove contaminants and promote long-term cleanup of soil or wastewater. *Arabidopsis halleri* (syn. *Cardaminopsis halleri*) is a model plant investigated for Zn and Cd hyperaccumulation; (Meyer et al. 2010; Zhao et al. 2006). However, Huguet et al. (2012) determined that mechanisms of Cd storage and detoxification in *A. halleri* differ from those that were previously found for Zn.

One of the main ways humans or animals are exposed to contaminants is through diet (Raad et al. 2014). Excessive intake of metals through diet can lead to their bioaccumulation in consumer tissues and numerous toxicities, e.g. hepatic and renal damage (Bulat et al. 2008; Järup et al. 1998; Nordberg 2004). The transfer of metals along food chains constitutes an important route of exposure that must be taken into account for ecotoxicological risk assessment (Hispard et al. 2008).The laboratory rat (*Rattus norvegicus* var. *alba*) is a common model organism used in toxicology. Numerous studies have used this model organism to assess the health risk of various substances (Brzóska et al. 2001;

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Cadková et al. 2013; Kumara et al. 2015). The aim of this work was to determine whether or not the hyperaccumulating plant *A. halleri* can affect element accumulation in the organism of laboratory rats (*Rattus norvegicus* var. *alba*) when added to feed.

Materials and Methods

This experiment was conducted during a 6 weeks period on 12 Wistar rats, each with an initial body weight of 150 g. During the experiment, each animal was placed in a metabolic cage (one animal per cage). The room housing the cages was equipped with air-conditioning. A constant temperature (22–24°C) and humidity level (approx. 70 %) were maintained at a constant day/night cycle (08:00 am–08:00 pm). Animals were given unlimited access to water. *A. halleri* aboveground biomass was sampled in the flowering stage in Pb, Cd, and Zn contaminated areas in the vicinity of Příbram city (Czech Republic), dried at laboratory temperature and homogenized.

Rats were randomly divided in two groups (six animals per group). Animals in the control group (C) were fed a commercially manufactured feed (ST-1) and the experimental group (P) was fed a mixture of ST-1 (60 %) and *A. halleri* (40 %). Trace element contents in the feed (ST-1) and mixture (60 % ST-1 + 40 % *A. halleri*) are presented in Table 1. The amount of added *A. halleri* (group P) was calculated in such a way that 25 g of feed contained 20 mg of zinc. Each individual was given 150 g of feed (6 × 25 g) per week, i.e. 120 mg Zn per week, i.e. 720 mg Zn per 6 weeks.

After exposure, rats were sedated and euthanized with a T-61 Euthanasia Solution injection (S: Embutramidum 200 mg, Mebezonii iodidum 50 mg, Tetracaini hydrochloridum 5 mg in 1 mL. PL: Dimethylformamidum, Aqua pro inj., Merck, Canada). Individual autopsies were carried out with Teflon instruments in order to obtain appropriate sample soft tissue (liver, kidney, testes, muscle, spleen and bone) for analyses. All tissues were weighed, properly washed in double distilled water, placed into Petri dishes and stored at −20°C until chemical analysis. Frozen tissues samples were dried by LYOVAC GT 2 (LEYBOLD-HERAEUS, GmbH, Germany) and microwave digested by lyophilisation using the mixture of hydrogen peroxide and nitric acid using MWS-3 + (Berghof Products + Instruments, Germany) as described in detail by Jankovská et al. (2011).

Concentrations of Cd in the digests were measured by Electrothermal Atomic Absorption Spectrometry technique using a Varian AA 280Z (Varian, Australia) with graphite tube atomizer GTA 120 and PSD 120 programmable sample dispenser. Concentrations of Cu, Fe, Mn and Zn in digests were determined by optical emission spectroscopy with inductively coupled plasma (ICP-OES) with axial plasma configuration using a Varian VistaPro, equipped with autosampler SPS-5 (Australia). Measurement conditions for all lines were power 1.2 kW, plasma flow 15.0 L min^{-1}, auxillary flow 0.75 L min^{-1}, and nebulizer flow 0.9 L min^{-1}.

The quality of analytical data was assessed by simultaneous analysis of certified reference material CRM 12-02-01 (Bovine Liver) (4 % of all the samples). Analytical data obtained for all determined elements were within in the confidence interval given by the producer. The background of the trace element laboratory was monitored by analysis of 15 % blanks prepared under the same conditions, but without samples, and experimental data were corrected by mean concentration of the elements in blanks, and compared with detection limits (mean ± 3 SD of blanks) which were 0.08 ng mL^{-1} for Cd, 0.7 ng mL^{-1} for Cu, 10.1 ng mL^{-1} for Fe, 2.7 ng mL^{-1} for Mn, and 7.5 ng mL^{-1} for Zn. Data of elements (Cd, Cu, Fe, Mn and Zn) tissue concentrations were analyzed with the two-sample t test for unpaired data. The differences were considered significant if $p < 0.05$. All computations used the program Statistica version 10. (Statsoft, USA).

Results and Discussion

Significant differences between Cd concentrations in the control (C) and *A. halleri* treated (P) groups were found in all tissues with the exception of bone tissue (Table 2). Differences between Zn concentrations in group P and C were significant only in the small intestine and kidney tissues (Table 2). The highest concentrations of Cd in the *A. halleri* treated group (P) were found in rat kidneys (23.013 μg g^{-1}) followed by the liver (3.455 μg g^{-1}). Remaining organs had the following concentrations in

Table 1 The trace element content (mg kg^{-1}) in the feed ST-1 and in mixture 60 % ST-1 + 40 % *A. halleri*

Feed	Cd	Cu	Fe	Mn	Zn
ST-1 (group C)	0	29.7	445	128	70
A. halleri	16.7	2.4	193	56.6	1950
60 % ST1 + 40 % *A. halleri* (group P)	6.7	18.8	344.2	99.4	822

Table 2 Metal concentrations ($\mu g\ g^{-1}$) in rat tissues

Metal	Group	Spleen	Muscle	Liver	Kidney	Intestine	Testes	Bone
Cd	C	$0.040^{b} \pm 0.035$	$0.009^{b} \pm 0.011$	$0.036^{b} \pm 0.013$	$0.061^{b} \pm 0.009$	$0.043^{b} \pm 0.027$	$0.007^{b} \pm 0.003$	0.099 ± 0.042
	P	$0.488^{b} \pm 0.200$	$0.023^{b} \pm 0.006$	$5.869^{b} \pm 2.909$	$23.013^{b} \pm 6.427$	$3.455^{b} \pm 3.264$	$0.269^{b} \pm 0.117$	0.132 ± 0.044
Zn	C	77.006 ± 17.508	44.886 ± 9.073	110.650 ± 22.626	$111.841^{b} \pm 8.315$	$102.198^{b} \pm 27.131$	205.331 ± 7.620	190.215 ± 9.724
	P	82.917 ± 9.026	57.237 ± 20.969	139.341 ± 45.696	$126.522^{b} \pm 15.255$	$168.960^{b} \pm 101.176$	186.816 ± 36.013	199.454 ± 17.232
Cu	C	5.561 ± 1.477	3.440 ± 0.957	14.182 ± 3.045	$27.261^{a} \pm 6.556$	$5.377^{a} \pm 1.419$	11.567 ± 0.521	0.659 ± 0.210
	P	6.149 ± 0.322	3.447 ± 0.473	19.589 ± 13.258	$34.274^{a} \pm 8.013$	$8.688^{a} \pm 4.930$	12.363 ± 1.934	1.025 ± 0.544
Fe	C	5702.108 ± 2390.460	80.586 ± 26.567	758.898 ± 199.726	$373.753^{a} \pm 67.863$	$68.989^{a} \pm 18.939$	166.712 ± 9.963	102.674 ± 17.041
	P	5665.636 ± 1107.787	106.597 ± 38.251	953.851 ± 458.227	$488.726^{a} \pm 125.197$	$117.523^{a} \pm 69.689$	209.871 ± 81.841	132.835 ± 65.618
Mn	C	$0.727^{a} \pm 0.189$	0.289 ± 0.098	7.851 ± 1.914	$3.186^{b} \pm 0.265$	2.382 ± 1.337	1.695 ± 0.097	0.315 ± 0.041
	P	$1.046^{a} \pm 0.318$	0.283 ± 0.100	6.888 ± 1.296	$2.497^{b} \pm 0.421$	3.283 ± 1.565	1.762 ± 0.386	0.293 ± 0.028

Numbers below are mean values ± SD of metal concentrations in individual organs of control (C) or treated (P) rat groups

[a, b] Indicate significant differences between compared groups: [a] $p < 0.05$, [b] $p < 0.01$

descending order: intestine, spleen, testis, bone and muscle. In the control group, Cd concentrations ranged from 0.099 $\mu g\ g^{-1}$ in bone to 0.007 $\mu g\ g^{-1}$ in the testes (Table 2).

Iron (Fe) concentrations in our experiment were highest in the spleen (Table 2); manganese (Mn) concentrations were highest in the liver (Table 2), and Zn levels were highest in the bones and testes (Table 2). Differences between other element concentrations in tissues of treated group P and those of control group C were significant only in the intestinal tissue for Cu and Fe (Table 2), in the kidney for Mn, Fe and Cu (Table 2) and in the spleen tissue for Mn (Table 2).

Zn is one of the most important trace elements in the biological system (Hambidge 2000). Approximately 85 % of Zn in the body is located in muscle and bone, and 11 % is found in the skin and liver (Jackson 1989). In both groups (P and C), bone tissues contained one of the highest concentrations of Zn; however, muscle tissues contained the lowest Zn levels. As an essential element, Zn is regulated through homeostatic mechanisms, and therefore, its content in the body is relatively stable (Welshe et al. 1994). King et al. (2000) reported that their study with laboratory rats proved the effect of homeostatic mechanisms, even at various Zn doses (10–100 mg kg^{-1}). This is partially supported by current results. Although there was a 12-fold increase in Zn levels in rat feed (group P) in the current study, Zn concentrations in the body tissues of both groups were very similar. Zn homeostasis can be disrupted by other elements, such as Cd (Ince et al. 1999). Both metals bind preferentially to the same proteins–albumin in the bloodstream and metallothionein (MT) and other proteins in tissues (Brzóska and Moniuszko-Jakoniuk 2001). Excessive intake of Zn and Cd stimulates the synthesis of cysteine rich MTs in the mucosa of the small intestine (Kägi 1991). Binding to MTs reduces the toxic effects of these two metals (Kelly et al. 1996). The higher affinity of Cd for MTs increases the concentration of free Zn^{2+} ions, which in turn stimulates the synthesis of other MTs (Funk et al. 1987). Swiergosz-Kowalewska (2001) reported that a Zn increase in the liver and kidneys is caused by Cd intoxication, and the author attributed this phenomenon to the higher synthesis of MTs, which, in the Cd_5Zn_2MT form, are passed from blood plasma to the liver and kidneys. A Zn increase in the liver and kidneys after Cd exposure was also described by Jihen et al. (2010). Furthermore, Chmielnicka et al. (1989) claimed that increased Zn content in the liver and kidneys can serve as indicators of ultrastructural damage caused by Cd intoxication. All of these findings are in agreement with current results. Higher Cd and Zn levels in group P feed resulted in higher concentrations of these metals in the small intestine and kidneys. The increase in Zn and Cd levels in these organs can be explained through the reduction of the toxic effects of Cd

and Zn homeostasis (Brzóska et al. 2001; Liu et al. 1992). Although a determination of MTs was not made in the current work, it is believed that the greater portion of both metals was bound to these proteins. Brzóska and Moniuszko-Jakoniuk (2001) summarize that the simultaneous administration of Cd and Zn reduces intestinal absorption of total Cd. Since the group C diet did not contain any Cd, this fact can be neither confirmed nor excluded.

In general, most of the dietary fiber comes from the cell walls of aerial plant parts (Selvendran 1984), and high fiber diet increases dietary cadmium intake (Järup et al. 1998). Since group C feed (ST-1) did not contain any Cd, *A. halleri* was the main source of Cd in this experiment. Furthermore, one of the most important metabolic parameters for Cd uptake is a lack of Fe. Low levels of Fe in the intestinal tract can increase Cd intake by up to 6 % (Flanagan et al. 1978). As described below, group P diet contained less Fe than did group C. After absorption in the gastrointestinal tract, the majority of Cd in the body is bound to MTs (Nordberg 2004). The kidneys are the primary target organ for chronic Cd exposure (Järup et al. 1998). A large portion of absorbed Cd is also stored in the liver and intestinal mucosa (Cooke and Johnson 1996). This is in accordance with current results, wherein rat kidneys from group P contained the highest Cd concentrations. The next two highest concentrations of Cd were measured in the liver and small intestine. The increase in Cd content in the small intestine, liver and kidneys can be attributed to an increase in the synthesis of MTs, which is stimulated by an increase in both Zn and Cd intake through feed. Furthermore, MTs have a strong binding affinity for Cu, which is even higher than that of Cd and Zn ions (Sabolic et al. 2010); it is believed that, despite the lower Cu status in the group P diet, the Cu increase in the kidneys and small intestine could be caused by an increase in MT synthesis.

Cd accumulation in testes was described for both inorganic and organic Cd forms (Bench et al. 1999; Haouema et al. 2008). In current experiments, Cd concentrations in the testes of *A. halleri* treated rats were 37 times higher than in unaffected rats. Previous studies indicated a reduction of testicular Zn (Bonda et al. 2004) and an elevation of testicular Fe (Maitani and Suzuki 1986) after Cd exposure. According to Bonda et al. (2004), Zn protects the testes against damage caused by Cd; however, in the case of an excessive amount of Cd in the body, the majority of Zn is bound to Cd-induced renal and hepatic MTs. Current results indicated a minor decrease in Zn and an increase in Fe between groups C and P. Nevertheless, current results did not detect any significant differences. The spleen is not a target organ for Cd toxicity; however, it accumulates Cd and suppresses both T cell and innate immunity (Demenesku et al. 2014). In the exposed group P, an increase in Cd spleen concentrations was noted. Even though an elevated concentration of Zn in the bone tissue was not observed, Kido et al. (1990) indicates that Cd intoxication can have a direct or indirect cause on bone damage. According to Chmielnicka et al. (1989), Cd accumulation in bone tissue can cause a decrease in Zn levels. The current study did not observe this trend. On the contrary, concentrations of Zn were higher in group P. Current results therefore confirm that Zn is an essential element for bones, and it can protect from Cd-induced bone damage when consumed in large amounts (Bulat et al. 2008).

Aside from Zn and Cd, the current study also measured Cu, Fe and Mn content. Stable tissue levels of these essential metals are maintained mainly through tight homeostatic control of intestinal absorption and biliary excretion (Hurley and Keen 1987; Kumara et al. 2015). However, Cd is known to impair these mechanisms (Chmielnicka et al. 1989; Noël et al. 2004; Schroeder and Nason 1974). Differences in Cu, Fe and Mn concentrations in the feed of both tested groups prevented determination of the influence of Cd and Zn on the metabolism of these biometals. In contrast to the feed dosage for control group C, the feed dosage for group P contained lower levels of Cu, Mn and Fe. With the exception of Mn, current results did not reflect this decrease. Determined concentrations were, in fact, higher in certain organs. Despite differences in Cu, Fe, and Mn feed concentrations, it is assumed that the increase in Cu and Fe levels in the kidneys and small intestine could be caused by an increased intake of Cd and Zn in the group P diet (Schroeder and Nason 1974). Nevertheless, it is essential to modify the experimental design in order to better understand the influence of *A. halleri* on Cu, Fe and Mn metabolism.

The aim of this work was to determine whether or not the hyperaccumulating plant *A. halleri* can affect element accumulation in the organisms of laboratory rats (*Rattus norvegicus* var. alba). In *A. halleri* leaves, Zn is bound mainly to malate or other organic acids (Sarret et al. 2009); Cd is also bound to organic acids, cell wall components and, to a lesser amount, thiol-containing molecules (Huguet et al. 2012). Earlier published papers indicated that plant-incorporated metals are often absorbed in greater amounts in comparison to their inorganic forms, which are artificially added to an animal diet (Cadková et al. 2013; Chan et al. 2004). To our knowledge, this is the first time *A. halleri* has been used in a feeding study. Current results suggest that using the hyperaccumulating plant *A. halleri* as a feed stresses the consumer organism not through its Zn content, but through its Cd content.

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Conflict of interest None.

Ethical standard All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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3.3 Ostatní publikace

1) Jankovská I, Brožová A, Matějů Z, Langrová I, Lukešová D, Sloup V. 2016. Parasites with possible zoonotic potential in the small intestines of red foxes (*Vulpes vulpes*) from Northwest Bohemia (CzR). Helminthologia **53**:290-293. https://doi.org/10.1515/helmin-2016-0028

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Research Note

Parasites with possible zoonotic potential in the small intestines of red foxes (*Vulpes vulpes*) from Northwest Bohemia (CzR)

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Summary

We determined the prevalence of primarily zoonotic parasites in the small intestines of 40 (20 males and 20 females) red foxes living near human dwellings. The total prevalence of parasite infection was 77.5 % (31/40); the prevalence was 37.5 % (15/40) for *Toxocara canis* and 35 % (14/40) for *Toxascaris leonina*. The mean intensity infection was 3 and 11 helminths for *T. canis* and *T. leonina*, respectively. The prevalence of other intestinal helminths and mean infection intensity in this study are given: *Echinococcus multilocularis* 40 % (16/40) with 1000 individuals, *Mesocestoides* spp. 40 % (16/40) with 8 individuals, *Uncinaria stenocephala* 10 % (4/40) with 8 individuals, and *Taenia pisiformis* 10 % (4/40) with 1 individual. With regards to prevalence and intensity of infection, as well as prevalence of individual parasites, there were no significant differences (P≥0.05) between male and female red foxes.

Keywords: red fox; human; zoonotic, parasite, infection

Introduction

The red fox (*Vulpes vulpes*) is a representative of the canid family. It is widely distributed in the Northern Hemisphere, and it is the most abundant wild carnivore living in the territory of the Czech Republic. Annual captures in the Czech Republic currently range between 60 000 – 80 000, and populations continue to grow (Placata, 2011). The increase in the distribution and density of the red fox in most European countries could be explained by a mortality rate reduction, which is due to an intensive campaigns of vaccination against rabies (oral baits) as well as to the opportunist behaviour of this species (Hanosset *et al.*, 2008; Červený *et al.*, 2004).

The red fox is a common host of several dangerous zoonotic parasites, primarily the tapeworm *Echinococcus multilocularis*, causative agent of alveolar echinococcosis, a parasitic disease that causes a severe hepatic disorder in humans (Letková *et al.*, 2006). Soil contaminated with red fox feces is a significant risk factor for zoonotic disease infection. Endoparasites (primarily *Echinococcus multilocularis* and *Toxocara canis*, but also *Uncinaria stenocephala*, *Mesocestoides* spp. and *Taenia* spp. tapeworms) or ectoparasites, such as fleas, ticks and mites (Kočišová *et al.*, 2006) of red foxes, can cause numerous health problems in humans and domesticated animals (mainly dogs).

Zoonotic roundworms (mainly *T. canis*) are not only present in their definitive hosts but also frequently occur in other animal species (Reiterová *et al.*, 2013), including humans. In these paratenic hosts, larvae do not develop into the adult stage, but rather migrate throughout the tissues and remain there as L3 arrested larvae for an extended period of time (Strube *et al.*, 2013).

Foxes live in close proximity to humans and domestic dogs, and this may have significant implications for public health. The aim of this study was to investigate the prevalence of zoonotic parasites in the small intestines of red foxes living near human dwellings.

Material and Methods

Forty (20 males and 20 females) red foxes were collected from an area surrounding the city of Karlovy Vary, in the northwest region of the Czech Republic (between October 2010 and March 2012). The study area covered approximately 6 km^2 with a fox density of 0.9-6 foxes/1km^2/year; average animal body weight was 5.5 kg (from 2.96 to 8.32 kg).

During necropsy, the small intestine was isolated and wrapped in plastic bags and frozen at -80°C until examination (app. one month) in order to inactivate the infective material. In order to detect intestinal helminths, a direct detection method was implemented using intestinal scraping or sedimentation techniques (ITS) (Tackmann *et al.*, 2006).

Helminths were identified according to size and morphology using two microscopes: an Olympus CX 21 and an Olympus *BX21*.

Statistical evaluation

Basic descriptive statistics were computed. The normality of the data was tested separately using a Shapiro-Wilks test. Considering the results of the normality test, a nonparametric Mann-Whitney U test was used to evaluate differences between males and females. Statistica ver. 12 (Statsoft, 2013) was used for all statistical analyses.

Table 1. Intestinal helminth prevalence and mean infection intensity in monitored red foxes

Species	Prevalence (%)	Mean intensity of infection (min – max)
Echinococcus multilocularis	40 (16/40)	1000 (32 – 3500)
Mesocestoides spp.	40 (16/40)	8 (4 – 123)
Taenia pisiformis	10 (4/40)	1 (1)
Toxascaris leonina	35 (14/40)	11 (1 – 96)
Toxocara canis	37.5 (15/40)	3 (1 – 24)
Uncinaria stenocephala	10 (4/40)	8 (1 – 12)

Results and Discussion

Parasitic contamination of urban and rural environments by Canidae excrements is a growing problem (Ondriska *et al.*, 2013; Papajová *et al.*, 2014). The presence of red fox feces in the soil of urban and suburban areas can threaten the health of animals and humans, mainly due to the presence of zoonotic parasite eggs (primarily *Echinococcus multilocularis* and *Toxocara canis*). We examinated the gastrointestinal tracts of 40 red foxes from the above-mentioned monitored area and found 77.5 % (31/40) of them to be infected by intestinal helminths. There were no statistically significant differences ($P \geq 0.05$) between male and female red foxes with respect to prevalence and intensity of infection and prevalence of individual parasites. The prevalence of intestinal helminths and their mean intensity is presented in Table 1.

Toxocariasis is a soil-transmitted helminthozoonosis, which is caused by *Toxocara* spp larvae infection in humans. Human infection is acquired through the ingestion of embryonated *Toxocara* eggs (eggs reach the environment via canid or felid stools and become infectious for humans and other hosts over the course of a few weeks). Dubná *et al.* (2007a) reported an 11.90 % prevalence of *Toxocara* eggs in 126 composite samples taken from child sandpits in Prague (Czech Republic). In paratenic hosts, such as humans and mice, hatched larvae migrate systematically throughout the body and have the ability to reach critical sites such as the eyes and central nervous system. Larvae of *Toxascaris leonina* can also invade the tissues of laboratory animals, and this species has the potential to cause human disease. Of the 42.5 % (17/40) of red foxes infected by roundworms (*T. canis* and/or *T. leonina*), 70.59 % (12/17) of them were infected simultaneously by both *T. leonina* and *T. canis*. Two of the red foxes (11.76 %) were infected with only *T. leonina* whereas three (17.65 %) were infected with only *T. canis*. The total prevalence of roundworms was 37.5 % (15/40) and 35 % (14/40) for *Toxocara canis* and *Toxascaris leonina* respectively. Letková *et al.* (2006) reported a 17.55 % prevalence of *T. leonina* and a 25.82 % prevalence of *T. canis* in red foxes (*V. vulpes*) from eastern Slovakia. Reperant *et al.* (2007) reported a lower prevalence of *T. leonina* in urban areas (8 %) in comparison to that of rural areas (59.6 %). This raises the question of whether rodent paratenic hosts play a major role in population dynamics of this species. Reiterová *et al.* (2013) reported anti-*Toxocara* antibodies in 6.6 % of small rodents trapped in Slovakia. Research carried out by Reperant *et al.* (2007) in Switzerland showed that as many as 59.6 % of foxes from rural areas were infected with *T. leonina* as opposed to only 8 % in urban areas. Reperant *et al.* (2007) found that *T. canis* and *T. leonina* co-occured in 14 % of the red fox population of Geneva, Switzerland. In our study, infection intensity ranged from 1 – 96 roundworms (*Toxocara* spp. and *T. leonina*) per fox. The co-occurence of *Toxocara* spp. and *T. leonina* in the definitive host is highly variable and is dependent on several factors: climate, environmental conditions, seasonal period and host age. This applies to infected wildlife as well as domestic animals (Okulewicz *et al.*, 2012). Antolová *et al.* (2004) reported a 47.1 % prevalence of *T. leonina* and an 8.1 % prevalence of *T.*

canis in red foxes from the Slovak Republic. Borecka *et al.* (2009) and Balicka-Ramisz *et al.* (2003) determined a 19.1 % and 39.8 % prevalence of *T. canis* in red foxes from Central and Western Poland respectively; they also determined a 0.0 % and 0.9 % prevalence of *T. leonina* in red foxes from Central and Western Poland respectively. Dubná *et al.* (2007b) monitored the prevalence of intestinal parasites in dogs from metropolitan Prague, rural areas surrounding Prague, and dog shelters in the Czech Republic; they reported *T. canis* in 6.2 % and 2.0 % of dogs in Prague and rural areas respectively; *T. leonina* was observed in 0.9 % and 1.7 % of canine faecal samples in metropolitan Prague and rural areas respectively.

In our study, we also observed the hookworm *Uncinaria stenocephala*, however, only 10 % (4/40) of foxes were infected with this helminth, with a mean infection intensity of 8 hookworms (Table 1). *Uncinaria stenocephala* is a nematode that belongs to the *Ancylostomatidae* family. Members of this family infect millions of people and animals worldwide. *U. stenocephala* is most pathogenic in dogs and other *Canidae*, which serve as the main hosts, and infection causes anemia or even death (Wasyl *et al.*, 2013). Rataj *et al.* (2013) reported *Uncinaria stenocephala* as the most frequently identified nematodes in red foxes from Slovenia.

Mesocestoides spp. are zoonotic cestodes of wild and domesticated carnivores. Although the adult tapeworms are relatively harmless intestinal parasites, the metacestode stages (tetrathyridia) can cause life-threatening peritonitis and pleuritis in several species including dogs, cats, non-human primates and, most likely, humans (Széll *et al.*, 2015). In our study, *Mesocestoides* spp. tapeworms were found in 40 % (16/40) of red foxes, with a mean infection intensity of 8 individuals (table 1). Széll *et al.* (2015) reported a similar prevalence of *Mesocestoides* spp. tapeworms in red foxes from Hungary (45.8 %).

Our study also revealed the presence of *Taenia pisiformis* (in 10 % 4/40 of red foxes), with a mean infection intensity of 1 individual (Table 1). The natural life cycle of *Taenia pisiformis* includes canines as normal definitive hosts and lagomorphs as typical intermediate hosts. *Cysticercus pisiformis*, the larval stage of *T. pisiformis*, can bring about economic impacts in rabbit breeding and cause serious health problems to the host, including liver lesions, digestive disorders, growth retardation, weight loss, and even death (Rajasekariah *et al.*, 1985). Rataj *et al.* (2013) reported a 2.1 % prevalence of *Taenia pisiformis* in red foxes from Slovenia.

Our study revealed a 40 % (16/40) prevalence of *Echinococcus multilocularis* - the most dangerous zoonotic parasite for man - with a mean infection intensity of 1000 individuals (Table 1). Alveolar echinococcosis is a rare human disease that is often lethal if left untreated (Szilágyiová *et al.*, 2015). It is caused by *Echinococcus multilocularis* at the larval stage. In Central Europe, the main definitive host of *E. multilocularis* is red fox. Red foxes from our monitored region (Northwest Bohemia) can wander into areas populated by humans, dogs and other domesticated animals, and can pose a significant threat to these inhabitants.

Conclusion

The tapeworm *Echinococcus multilocularis* and roundworm *Toxocara canis*, which possess severe zoonotic potential, were observed in red foxes from the monitored area. For this reason, these individuals were treated monthly with anthelmintic bait (50 bait pieces/km^2) for an entire year following the study.

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The effect of *Syphacia muris* on nutrient digestibility in laboratory rats

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Abstract

This study was carried out to investigate how pinworm infection in rats affects nutrient digestibility in the hosts. Twenty-four male outbred Wistar rats were randomly divided into two groups of 12 rats each. The rats from the first group (GI) were kept in cages with bedding containing pinworm eggs, and the second (control) group (GII) were kept in a separate room in clean, uncontaminated filter-top cages. The animals were put into individual metabolic cages later. Metabolic trials lasted five days and records of animal weight, food ingestion, and faecal weight were taken daily. Based on laboratory analysis of the feed and faecal nutrient content, digestibility values were determined. On day 15 of the experiment, the animals were euthanized. Although *Syphacia muris* were found in all rats from the GI group, animals exhibited no clinical signs. In our experiment, *S. muris* infection reduced the overall digestibility of all measured nutrients ($P < 0.01$). The most significant differences in digestibility were observed in the case of crude fibre and mineral matter ($P < 0.01$).

Keywords

laboratory rat, *Syphacia muris*, infection, nutrient, digestibility

Rats are the world's most frequently used laboratory animals. They are used for scientific as well as for a variety of commercial purposes. They are easy to keep, and their breeding technology is very sophisticated. Nevertheless, rats suffer from many parasitic diseases, which can influence experimental results.

One of the most common parasites found in breeding rats is the pinworm. Due to their biology, direct development, short embryonic period, and incidence of autoinfection, pinworms are a very prolific group of parasites. In laboratory rats, the most commonly found pinworm species are *Syphacia muris* and *Aspiculuris tetraptera*.

Although pinworm parasites of laboratory rodents are considered to be relatively non-pathogenic and infections are generally regarded as symptomless, there have been reports of laboratory rodents with a variety of conditions such as intestinal impaction, intestinal intussusception, mucoid enteritis, necrosis in all layers of the intestinal wall, and rectal prolapse. These conditions are thought to be associated with heavy pinworm burdens.[1–6]

One area in which rats are often utilized is feed testing. Rats are most commonly used in experiments designed to test the digestibility of individual nutrients in various feeds. However, it is very difficult to keep rats free of pinworm infection,[7,8] and therefore, many of these experiments are conducted with infected animals.

The aim of this study is to investigate how pinworm infection affects nutrient digestibility. We monitored

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feed intake, nutrient digestibility and feed conversion in laboratory rats with *S. muris* infection.

Materials and methods

Animals and procedure

Twenty-four male outbred Wistar rats were obtained from the specific pathogen-free (SPF) rat colony of Charles River Laboratories, Sulzfeld, Germany. The animals had an average weight of 89 g (4 weeks old). They were randomly divided into two groups of 12 rats each. The rats from the first (experimental) group (GI) were kept in the cages with bedding containing pinworm eggs for 10 days. Contamination was achieved by adding bedding from the cages of *S. muris* infected rats to the breeding containers. After this 10-day period, *Syphacia* eggs in all the animals of GI were detected using a cellophane-tape test. The second (control) group (GII) was kept in a separate room in clean, uncontaminated filter-top cages for 10 days; the beddings of GII group were thoroughly sterilized.

The rooms were maintained at 22 ± 2°C and 55 ± 5% relative humidity, with normal 12:12 h light dark cycles and constant air circulation. All animals were monitored daily for the presence of pinworm eggs using the cellophane-tape test.

Thereafter, the animals were kept in individual metabolic cages (Tecniplast, Buguggiate, Italy) with normal 12:12 h light dark cycles and constant air circulation, and room temperature was kept at 24 25°C and 50 ± 5% relative humidity. The animals from the control group (GII) were placed in individual metabolic cages in a separate experimental room. The diet composition (ST-1; Bergman, Kocanda, Czech Republic) is shown in Table 1. The rats were fed this diet ad libitum.

Table 1. Composition of rat diet (ST-1 Bergman).

Ingredients	Unit	
Crude protein	%	25.5
Ash	%	6.26
Dry matter	%	86.5
Crude fibre	%	4.3
Nitrogen-free extract	%	47.7
Fat	g/100 g	3.76
Calcium (Ca)	mg/kg	12700
Potassium (K)	mg/kg	10500
Magnesium (Mg)	mg/kg	2210
Sodium (Na)	mg/kg	1780
Phosphorus (P)	mg/kg	8110

Metabolic trials lasted five days, and records of animal weight, food ingestion, and faecal weight were taken daily. Daily faecal outputs were collected in plastic bags, weighed, thoroughly mixed and stored at −20°C for later analysis.

From these measured values, we calculated daily weight gain, feed intake and feed conversion. Based on laboratory analysis of the nutrient content of feed and faeces, digestibility values were determined.

On day 15 of the experiment, the animals were euthanized using an intravenous administration of T-61 solution (Intervet, Boxmeer, The Netherlands). The viscera (stomach, small intestine, colon and caecum) of each rat were collected and processed for worm recovery, enumeration and identification.

All of the experimental procedures were conducted in accordance with Czech legislation (section 29 of Act No 246/ 1992 Coll., on the protection of animals against cruelty, as amended by Act No 77/2004 Coll. and Directive 2010/63/EU on the protection of animals used for scientific purposes and the guidelines and recommendations of the Federation of European Laboratory Animal Science Associations).

Laboratory analysis

The food and faecal samples were lyophilized (LP3 lyophilizer; Jouan, France) and grounded (Cyclotec 1093, Tecator, FOSS, Hillerød, Denmark) in order to pass through a one-millimetre stainless steel screen. The feed and faecal dry matter contents were determined at a temperature of 103°C (UFB500, Memmert). The Kjeldahl method (Kjeltec 2400, Foss) was used to determine crude protein (CP) levels, and crude fibre was measured using the Henneberg and Stohmann method (Coex 106, VELP Scientifica, Usmate, Italy). Fat digestibility was analysed with the help of the Soxhlet method (SER 146/8, VELP Scientifica). This method is based on the principle of extraction with an organic solvent (petroleum ether). During this extraction the results include fat and any other substances which are readily soluble. The content of other substances, such as carotenoids, chlorophylls, cholesterol and fat-soluble vitamins, were not taken into account. Gross energy was measured using an LS10-A calorimeter (Laget, Prague, Czech Republic), and ash content (mineral matter) was determined at 550°C (LH 15/13; LAC, Rajhrad, Czech Republic). The ash samples were incinerated, then boiled in 6 M hydrochloric acid and the insoluble residue was filtered through an ashless filter, which was dried and burned. Analyses were performed according to EC No. 156/2006 modified to a specific device.

Values of nitrogen-free extract (NFE) were calculated as follows: 100 (% moisture + % protein + %

fibre + % ash + % fat). Nutrient digestibility was defined as the difference between food intake and faecal excretion expressed as a percentage of the intake: 100 {[(feed insoluble ash × faecal nutrient)/(faeces insoluble ash × feed nutrient) excretion)/intake] × 100}.

Data analysis

The mean and standard deviation for each group were calculated and compared using analysis of variance (ANOVA), with the Tukey Kramer test to evaluate differences among the groups.

Results

Syphacia muris were found in all the rats from the GI group (Table 2); GII rats were free of these parasites. All the rats in the experiments remained in good health, with no clinical signs or visible changes in the mucosa, no alterations in the structure of faeces, and no signs of diarrhoea. Therefore, pinworm infection could be generally regarded as subclinical. In the current experiment, *S. muris* infection reduced the overall digestibility of all measured nutrients ($P < 0.01$) (Table 3). The feed intake of the healthy animals was higher than that of the infected animals, and the difference was statistically significant (Table 4).

Table 2. Number of *Syphacia muris* individuals in the rats of the GI and GII groups.

	GI			
		Adults		
	Larvae	Male	Female	GII
1	450	78	130	0
2	1080	73	71	0
3	740	47	53	0
4	720	263	215	0
5	350	32	22	0
6	680	19	21	0
7	400	73	44	0
8	590	127	109	0
9	280	95	81	0
10	1220	96	89	0
11	670	223	187	0
12	690	128	187	0

GI: Rats from the first (experimental) group were kept in the cages with bedding containing pinworm eggs for 10 days. GII: Control group rats were kept in a separate room in clean, uncontaminated filter-top cages for 10 days.

The most significant differences in digestibility were observed in the cases of crude fibre and mineral matter ($P < 0.01$). Crude fibre digestibility in the uninfected rats was very high ($36.95 \pm 10.28\%$), whereas crude fibre digestibility values in the animals with pinworms averaged $23.88 \pm 3.03\%$. Similarly, mineral matter retention was significantly lower in the infected animals ($59.46 \pm 4.62\%$) than in the control rats ($82.95 \pm 1.88\%$). The values of nutrient digestibility are shown in Table 3 and growth parameters are presented in Table 4.

Discussion

Rats are among the most important laboratory animals. They are used for a variety of experiments, including metabolic trials and food testing. Unfortunately, pinworm infection is not taken into account in many of these assays. Although these parasites are considered non-pathogenic, pinworms definitely affect their hosts in a variety of ways. Wagner[9]

Table 3. Average values of nutrient digestibility of monitored rat groups (%).

	Uninfected	Infected
Ash (mineral matter) digestibility	82.95 ± 1.88	59.46 ± 4.62*
Crude protein digestibility	93.94 ± 1.00	87.19 ± 1.76*
Fat extract digestibility	94.79 ± 1.51	86.53 ± 1.86*
Crude fibre digestibility	36.95 ± 10.28	23.88 ± 3.03*
Organic matter digestibility	94.07 ± 1.01	85.13 ± 1.64*
Nitrogen-free extract digestibility	95.54 ± 1.82	87.58 ± 1.52*

*Significantly different from control group ($P < 0.01$).

Table 4. Growth parameters of monitored rat groups.

	Uninfected	Infected
Initial weight (g)	224.08 ± 10.40	198.37 ± 6.20†
End weight (g)	290.96 ± 12.72	237.95 ± 11.14†
Daily gain (g)	16.72 ± 1.76	9.90 ± 1.42†
Feed conversion gain (g*)	1.75 ± 0.17	2.34 ± 0.21†
Daily feed intake (g)	29.05 ± 0.99	22.92 ± 1.75†

*Feed intake (g) per gram weight gain.
†Significantly different from control group ($P < 0.01$).

reported that uninfected rats grew faster and attained weights (at 6 weeks) that were, on average, 12% higher than those of their infected counterparts. The competition for and utilization of the host nutrients are likely explanations for growth depression.[10] Pinworms cause changes in haemopoiesis,[11] affect lymphocyte proliferation[12] and reduce water and electrolyte transport in the intestine.[13]

The results of this study show that pinworms also negatively affect the digestibility of all evaluated nutrients. We compared parasitized rats to non-parasitized rats given access to the same amounts of food, so that the consequences of parasitism on food utilization could be directly quantified and evaluated. Statistically significant differences were observed between groups GI and GII with respect to digestibility of all tested nutrients.

The effects were most marked for crude fibre and mineral ash. This is a consequence of the fact that pinworms are located primarily in the caecum, where fermentable polysaccharides act as an energy source for microorganisms, and where mineral absorption takes place. Similar results have been noted in pigs infected with the nematode *Oesophagostomum dentatum*, which is localized in the colon; and all of these digestibility coefficients measured were affected by this parasite. However, the effects were most marked for crude fibre and mineral ash.[14] Similar changes were also reported in studies by Kaarma.[15] Munger and Slichter[16] reported significantly low dry matter digestibility in kangaroo rats *(Dipodomys microps)* with *Trichuris dipodomys* infection.

The role of the large intestine in digestion has not been fully documented. In rats this is mainly where bacteria degrade the fermentable part of fibre to short-chain fatty acids, which are sources of energy and are precursors in the synthesis of glucose and body fat, and which also play an important role in various physiological functions.[17] The main site of fibre degradation in rats is the caecum, and in this study, 98% of all pinworm populations were found in this region. Although pinworms did not cause visible clinical symptoms or changes in the mucosa, caecum function was likely to have been affected. At the end of the experiment, we found 404 to 1405 individual *S. muris* nematodes in one rat!

Most studies dealing with nutrient digestibility in relation to parasitic infection have been carried out on ruminants infected with nematodes, which are located in the abomasum and small intestine.[18–23] The interaction between intestinal helminth infection and nutrition has been reviewed by Poppi et al.,[24] Van Houtert and Sykes,[25] Coop and Holmes,[26] Coop and Kyriazakis,[27] Petkevičius,[28] and Holmes et al.[29] In general, gastrointestinal nematodes reduce nutrient availability in the host through reductions in both voluntary feed intake and/or absorbed nutrient efficiency. The degree to which these two mechanisims impair production is, to some extent, dependent on the species of parasite and its location in the gastrointestinal tract.[27,30]

The key feature of gastrointestinal nematode infection is an increased loss of endogenous protein to the gastrointestinal tract,[31–34] partly as a result of plasma protein leakage, increased mucoprotein production and sloughing of epithelial cells in the alimentary tract.[32] Poppi et al.[31] and Kimambo et al.[35] have suggested that the amount of non-reabsorbed endogenous nitrogen that leaves the terminal ileum may be considerable (up to 4 5 g of nitrogen per day). In this study, high protein digestibility was observed in both rat groups. The main reason for this was the feed composition, which contained large proportions of fish; as a result, both groups of rats grew very quickly. However, there was also a statistically significant difference in CP digestibility in the control group. This may have been due to limited caecum function as well as to reduced absorption or utilization of ammonium nitrogen in other forms, which also takes place in the caecum.[36]

The most significant effect that gastrointestinal parasitism has on its host is voluntary feed intake depression.[22,23,37,38] Large acute infections result in significant decreases in the feed intake of parasitized animals;[39] however, a degree of inappetence is present even in subclinical infections.[28] Several hypotheses have been postulated for this reduction in voluntary feed intake, such as alterations in amino acid availability, changes in flow rates and pH of digests, alterations in gut peptides and hormones, and direct neural effects on the central nervous system.[28,40] Even in this current study, the feed intake of healthy animals was higher than that of infected animals, and the difference was statistically significant.

Recently, the influence of nutrition on gastrointestinal parasites has been examined from different perspectives. Some studies show that reductions in voluntary feed intake, as measured by a depression of dry matter intake, are observed only during primary infection.[22,23] Other studies have shown that adding protein to the animal diet improves resistance and resilience to several nematode infections.[21,28,41] In recent times, the influence of host nutrition on helminth populations has also received attention, and much information is available.[28,42–47] Gastrointestinal helminths have very specific physicochemical requirements of their host gut environment, and nutritionally mediated changes may have a direct influence on parasite populations.[48]

The possible effects that varying levels of dietary fibre intake have on the occurrence of endoparasitic infection in pigs was reported in the study by Pearce,[49] in which the use of grower diets high in

non-starch polysaccharides was associated with an increased risk of Trichuris infection.

In our study, *S. muris* infection caused no visible signs; no visible changes in the mucosa, no alteration in faecal structure, and no diarrhoea. However, pinworms definitely affect their hosts in different ways. Pinworms alter the gastrointestinal tract environment as well as nutrient digestibility, and they most likely also affect the bacterial community structure, including possible secondary bacterial infections.A secondary bacterial infection would likely lead to impaired absorption and an increase in the flow of materials.[50] Pattison et al.[14] have reported that a high flow rate of digesta in the large intestine would reduce the duration of bacterial action on dietary fibre, thus accounting for a reduction in digestibility.

In conclusion, rats infected with *S. muris* are not suitable for experiments designed to test nutrient digestibility in various feeds, because these parasites significantly reduce nutrient digestibility of individual nutrients ($P < 0.01$). It is difficult to understand the mechanisms involved in these reductions, but it has been suggested that subclinical parasitism may affect the nutrition of the host, particularly through reduced absorption, an increased flow rate of digesta, and reduced enzyme activity.[14,28] Further detailed investigations would be beneficial.

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Declaration of conflicting interests

On behalf of all authors the corresponding author states that there is no conflict of interest.

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Compliance with ethical standards

All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic Act No. 246/1992 coll. on the protection of animals against cruelty and EC Directive 86/609/EEC.

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RESEARCH ARTICLE

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Fanniidae (Diptera): new synonym, new records and an updated key to males of European species of *Fannia*

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Abstract

Based on revision of large recent collections of the authors, the following five species are first recorded from the Czech Republic: *F. collini* d'Assis-Fonseca, 1966 (simultaneously first record in Central Europe), *F. lugubrina* (Zetterstedt, 1838), *F. melania* (Dufour, 1839), *F. slovaca* Gregor & Rozkošný, 2005, and *F. brinae* Albuquerque, 1951 (simultaneously first record from low altitudes). Another species, *F. alpina* Pont, 1970, is first recorded from Slovak Republic, and *F. cothurnata* (Loew, 1873) is first recorded from Kazakhstan. An updated key to males of European species of *Fannia* is presented. A list of Czech and Slovak Fanniidae is appended. One new synonym is established: *F. lucida* Chillcott, 1961 is considered junior synonym of *F. norvegica* Ringdahl, 1934. Altogether two species are first recorded from Bohemia [*F. cothurnata* (Loew, 1873) and *F. vespertilionis* Ringdahl, 1934] and three for Moravia [*F. alpina* Pont, 1970, *F. conspecta* Rudzinski, 2003, and *F. limbata* (Tiensuu, 1938) – this species considered in Central Europe very rare was found in numbers near waters both running and standing in early spring under unusually warm temperature conditions].

Keywords

Diptera, Calyptrata, Fanniidae, Europe, Czech Republic, Slovak Republic, Kazakhstan

Introduction

The Fanniidae are a small family of Calyptratae distributed worldwide, comprising more than 360 extant species (Pape et al. 2011) in 5 genera (*Euryomma*, *Fannia*, *Piezura*, *Australofannia*, *Zaelandofannia*). In Europe, 85 species are known (Pont 2007, Rudzinski 2003, Gregor and Rozkošný 2005). Some representatives are known from their forensic, medical and hygienic importance. Several species have a tendency for synanthropy. Females are attracted to decaying organic matter, often in great numbers. In addition, males are attracted to the same substrate but much less frequently. In our (unpublished) experiments with pig carcasses, almost 20 000 specimens were collected and females were about 13 times more frequent.

Adults may be distinguished from representatives of all other families of calyptrates by an asetose meron, the second anal vein strongly bent towards the first anal vein, so that prolongation of it will cross first anal vein at most at the wing margin, the scutellum without setulae on the lower surface, and the Sc vein having only one (basal) bend. Moreover, females lack crossed interfrontals and proclinate orbitals.

Larvae are aquatic to terrestrial, often living in semi-aquatic media. Larvae and puparium of fanniids are readily identifiable by sharing a dorso-ventrally flattened body, characterized by conspicuous feathery, forked, tufted, or button-like processes distributed over most of the dorsal and lateral surface of segments (and in reduced form also on ventral surface). An interesting character known at least in *Fannia canicularis* is a trichoid sensillum on the posterior spiracular plate, representing a sensory organ otherwise unknown in the Calyptratae (Grzywacz et al. 2012, Domínguez and Pont 2014).

For more details about morphology, biology, and zoogeography of the family see Chillcott (1961), Rozkošný et al. (1997), Pont (2000) or Domínguez and Pont (2014).

In the last 10–15 years, we collected some 200 000 specimens of Fanniidae mostly by means of mass collecting methods (Malaise traps, pyramidal traps exposed above pig carcass or heap of decaying wood, protein traps, yellow and white water pan traps, etc.) and stored them in ethyl alcohol. Using morphospecies method (based chiefly on examining male genitalia) we selected some 3 000 specimens which were dry mounted and identified to species. This revealed many important findings and the results of our studies are published herewith.

Material and methods

This paper is based on extensive materials of Fanniidae deposited in the collection of the Czech University of Life Sciences, Prague (CULSP) and partly in the collection of the North Bohemian Museum, Liberec (NBML) and Institute of Criminalistics, Prague (ICP). Some specimens originate from the Canadian National collection of Insects and Arachnids, Ottawa (CNC), Natural History Museum, London (NHM), National Museum, Prague (NMP) and Moravian Museum, Brno (MMB).

The identification of the Central European species is possible using the keys in the review of the European species (Rozkošný et al. 1997), which also summarises all the available data on the morphology of immature stages and adults, development and biology, medical, hygienic and economic importance, and distribution. More recently Pont (2002) has proposed some new synonyms based on a study of Zetterstedt´s types. Two recently described species, *F. conspecta*: Rudzinski (2003) and *F. slovaca*: Gregor and Rozkošný (2005), are lacking in the above mentioned keys. So we elaborated an updated key to males of European species of *Fannia*. In order to make our updated key more convenient for users, the couplets from Rozkošný et al. (1997) have ben maintained mostly unchanged, including reference to figures in that publication.

Distributional records are taken mainly from Pont (2007) if not stated otherwise.

Figure preparation: genitalia together with 2–3 pregenital segments were removed and macerated in potassium hydroxide solution (approx. 10%) in small vials submerged in hot water for 1–2 hours. After neutralizing with 8% acetic acid, the genitalia were dissected in glycerine and their parts (without hypandrium) photographed by means of an Olympus E-41 digital camera mounted on an Olympus BX51 compound microscope. Images were edited with the computer software Quick Foto micro 2.3 provided with Deep focus 3.1. Each image resulted usually from combining 7–15 layers. Images were improved by means of Adobe Photoshop.

Microphoto (Fig. 14) was preperaed by means of ZEISS Ultra Plus SEM operating at low accelerating voltage of 5 kV. A chamber secondary electron detector was used for imaging in topographical contrast. Before the analysis the sample was sputter-coated with 3 nm of platinum to obtain electrically conductive surface.

Abbreviations used: MT = Malaise trap, SW = sweeping, ET = emergence trap.

Results

(species are arranged alphabetically)

Fannia alpina Pont, 1970. Material examined (2♂): 1♂, Slovakia b., V. Tatry Mts, Tatranská Lomnica - 3 km NW, 49°10'N, 20°15'E, 1100 m, 13.viii.1982, M. Barták; 1♂, Moravia bor., Beskydy, H. Lomná, Hruška, 49°30'29"N, 18°36'56"E, 23.v.-19.vi.1999, MT, M. Barták (- all CULSP). Broadly distributed (Palaearctic and Oriental region) but uncommon species, in Europe previously known from Austria, the Czech Republic and Finland. It has also been found in Japan (Nishida 1974) and Nepal (Nishida 1994). First record from Slovak Republic and Moravia.

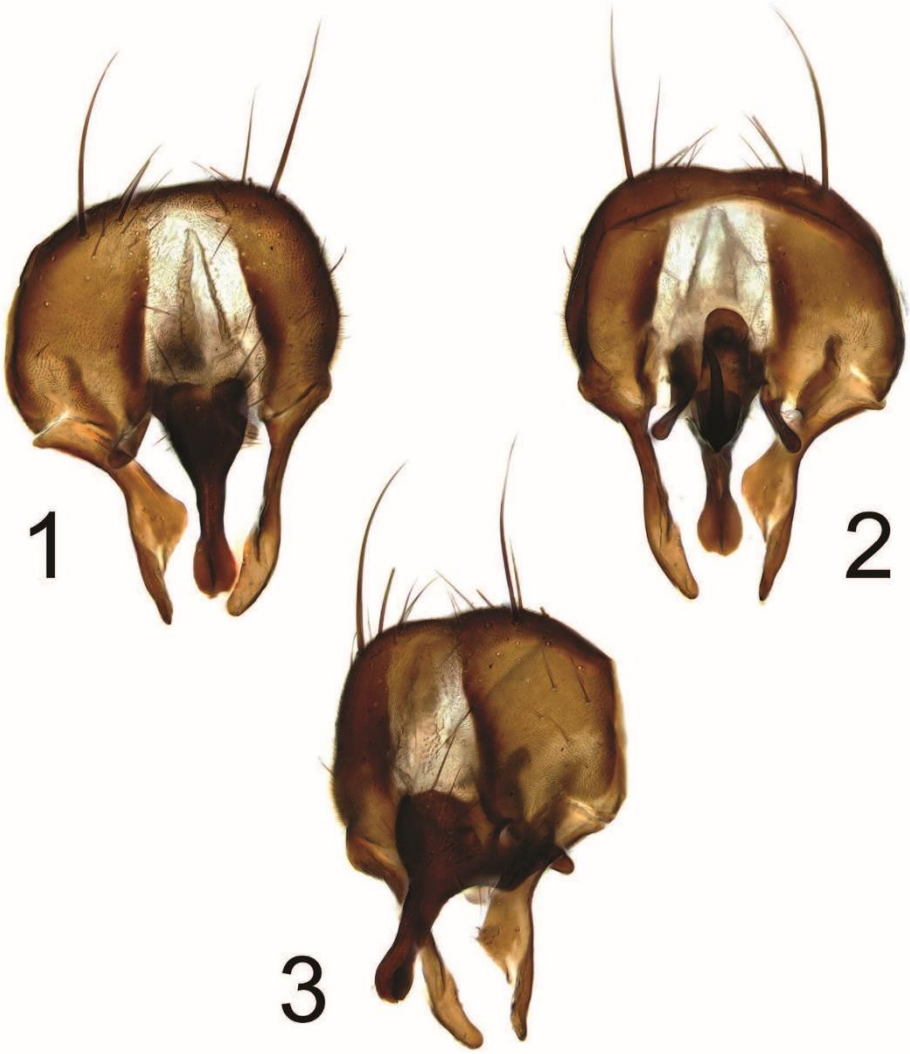
Fannia brinae Albuquerque, 1951. Material examined: 1♂, Moravia mer., Hustopeče, 240 m, alfalfa, conventional agric., 45°57'39"N, 16°41'49"E, 1.-30.vii.2008, ET, J. Rotrekl (CULSP). Very rarely collected species known up to now only from a few localities in French Alps. Not only essential characters for recognition of the species (broad frons with developed orbitals and very long submedian anterodorsal and dorsal seta inserted close together on the same level) but also all other charac-

ters of the above specimen even in small details agree with redescription by Gregor and Rozkošný (1993) except the following: 7 pairs of strong orbitals present (with small hairs between them), uppermost one strong and lateroclinate and abdomen with narrow dark midline. The specimen possesses several characters mentioned in this species (and also in allied species, *F. altaica*) by Pont and Vikhrev (2009): several fine setulae present on upper part of parafacials (possibly a variable character), only a single small seta in addition to strong seta on both proepisternum and proepimeron and bare propleural depression, but contrary to this paper, fore tibia of our specimen has distinct (even if short) anterodorsal seta. Moreover, pedicel seems to be paler (reddish-brown) anteriorly near apex. First record for the Czech Republic and the first record from low altitudes.

Fannia carbonaria (Meigen, 1826) (Figs 1–3). Material examined (4♂): 1♂: Bohemia b., Krkonoše, Bíner, 609 m, damp meadow, 50°37'50"N, 14°40'34"E, 21.v.-16.vi.2009, MT, J. Vaněk; 1♂: same data but 16.vi.-7.vii.2009 (- all CULSP); 1♂: Slovakia, Dvorčany, 16.iv.1957, J. Čepelák (MMB); 1♂: Kazakhstan, Almaty reg., Kazstroj, 1240 m, 43°17'26"N, 77°18'22"E, 7.-21.v.2013, MT, O. Nakládal (CULSP) – first record from Kazakhstan. Broadly distributed Holarctic species (also in Taiwan), but everywhere apparently rare. World species of *carbonaria* subgroup have been keyed by Wang et al. (2009), but mid tibia of *F. carbonaria*, stated here as having only 1 posterodorsal has in fact mostly at least 2 such setae (number varying from 1 to 5); also couplet 7 of their key is confusing because *F. carbonaria* has white squamae. Also in the key by Rozkošný et al. (1997) is this species wrongly arranged because it has no long posteroventrals at least on apical half of hind femur.

Fannia collini d'Assis-Fonseca, 1966. Material examined: 1♂, Bohemia b., Frýdlantská pahorkatina Hills, Poustecká obora nr. Poustka, 50°57'33.6"N, 15°3'50.9"E, 18.vii.-8.viii.2012, MT, J. Preisler & P. Vonička (NBML). The species has been known previously only from Great Britain. Our specimen agrees in nearly all details with original description incl. very distinctive genitalia. Slight differences are as follows: 12 pairs of orbital setae (and not „8-10", as stated in the original description) and anterodorsal seta on t3 is very short and fine (and not „strong"). Males of *F. collini* may be easily identified using key in Rozkošný et al. (1997), female remains unknown. First record for the Czech Republic and in Central Europe.

Fannia conspecta Rudzinski, 2003. Material examined (10♂): Bohemia c., Praha Troja, 184 m, 50°7'15"N, 14°23'53"E, emergence trap baited with pig carcass, 1 ♂: 2.-9.v., 1♂: 15.-22.v., 2♂: 22.-29.v., 1♂10.-17.vii., 1♂: 17.-24.vii., 2♂: 4.-11.ix. - all 2012, M. Barták & H. Šuláková (CULSP); 1♂ Bohemia c., Mníšek pod Brdy, 8.viii.2012, 49°52'10"N, 14°15'38"E, 385 m, ex larva, from a human corpse, H. Šuláková; 1♂: Moravia, Hornomoravský úval, Kroměříž, nr. Mošténka brook, 49°19'50"N, 17°23'10"E, 205 m, protein trap (chicken meat), H. Šuláková, 11.i.-20.iii.2010 (ICP). This species was known previously from the Czech Republic (Bílina – Jirásek III, 50°33'35"N, 13°47'44"E, 310 m, MT, Phragmitetum, 14.v.–23.vii.1998, M. Barták), Germany, Denmark, Portugal, Greece and South Russia



Figures 1–3. *Fannia carbonaria* (Meigen, 1826), hypopygium: **1** dorsal view **2** ventral view **3** oblique view.

(Grzywacz and Prado e Castro 2012). Additional records of this uncommon species from the Czech Republic were found and first records from Moravia.

Fannia cothurnata (Loew, 1873). Material examined: 1♂, Bohemia mer., Vráž nr. Písek, 400 m, nr. brook, 49°23'59"N, 14°7'58"E, 24.v.-24.vi.2010, MT, M. Barták; 1♂, Kazakhstan Almaty reg., Kazstroj, 1240 m, 43°17'26"N, 77°18'22"E, 7.-21.v.2013, MT, O. Nakládal (- all CULSP). Broadly distributed in Europe and Near East. In the Czech Republic published previously only from Moravia (Rozkošný and Gregor 1988). First records for Bohemia and Kazakhstan. The

specimen from Kazakhstan has only one each antero- and posterodorsal seta on mid tibia but otherwise corresponds in all details to typical form.

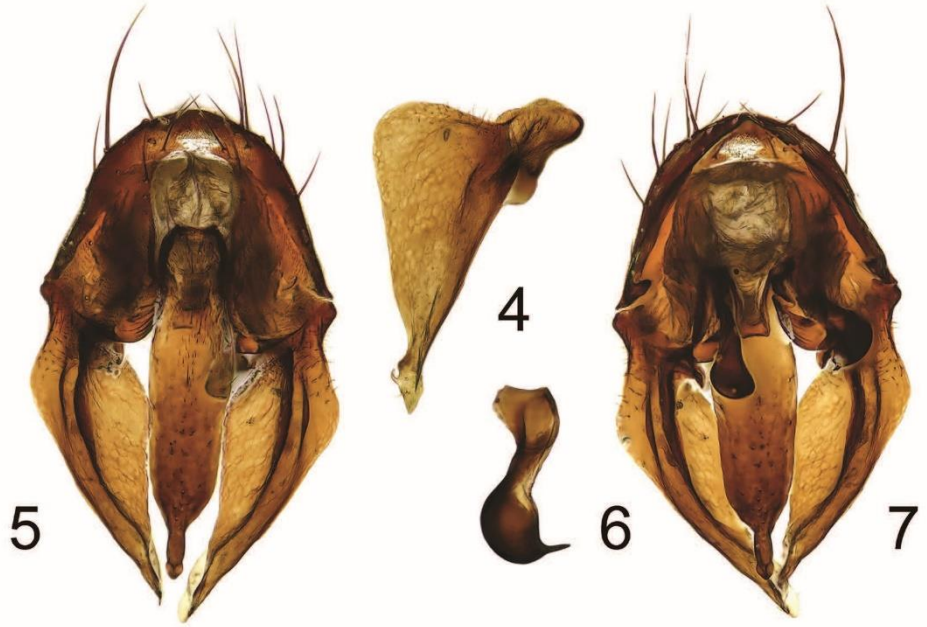
Fannia limbata (Tiensuu, 1938). Material examined (13 ♂): 10♂: Moravia occ., Jihlava-Pávov, 495 m, 49°26'26"N, 15°35'44"E, wetland nr. pond, 16.iv.-3.v.2009, MT, M. Barták; 3♂: Bohemia b., Děčín-Čertova voda, right Labe shore, 130 m, 50°48'47"N, 14°13'35"E, MT baited with decaying meat, 11.-25.iv.2009, M. Barták (all CULSP). Rarely collected species known only from Scandinavia, Germany and the Czech Republic (previously one record only from Kostelní Lhota nr. Sadská). First record from Moravia and only the second from Bohemia. All Czech records originate from the vicinity of water (both running and standing) under unusually hot early spring conditions.

Fannia lugubrina (Zetterstedt, 1838). Material examined: 1♂, Bohemia b., Krkonoše Mts, Labská rokle nr. Labská bouda, 1300 m, 50°46'19"N, 15°32'43"E, 31.v.-15.vi.2007, MT, J. Vaněk (CULSP). A Holarctic species, in Europe distributed in Scandinavia and North Russia and several temperate European countries: Belgium, Austria, and Poland. First record for the Czech Republic.

Fannia melania (Dufour, 1839). Material examined: 2♂: Bohemia b., Jizerské hory Mts, Holubník Mt., Bílé Buková, 900 m, 50°49'57"N, 15°10'51"E, 16.vi.-14.vii.2011, MT, J. Preisler & P. Voníčka (NBML, CULSP). Broadly distributed but apparently rare Eurasian species. First record for the Czech Republic.

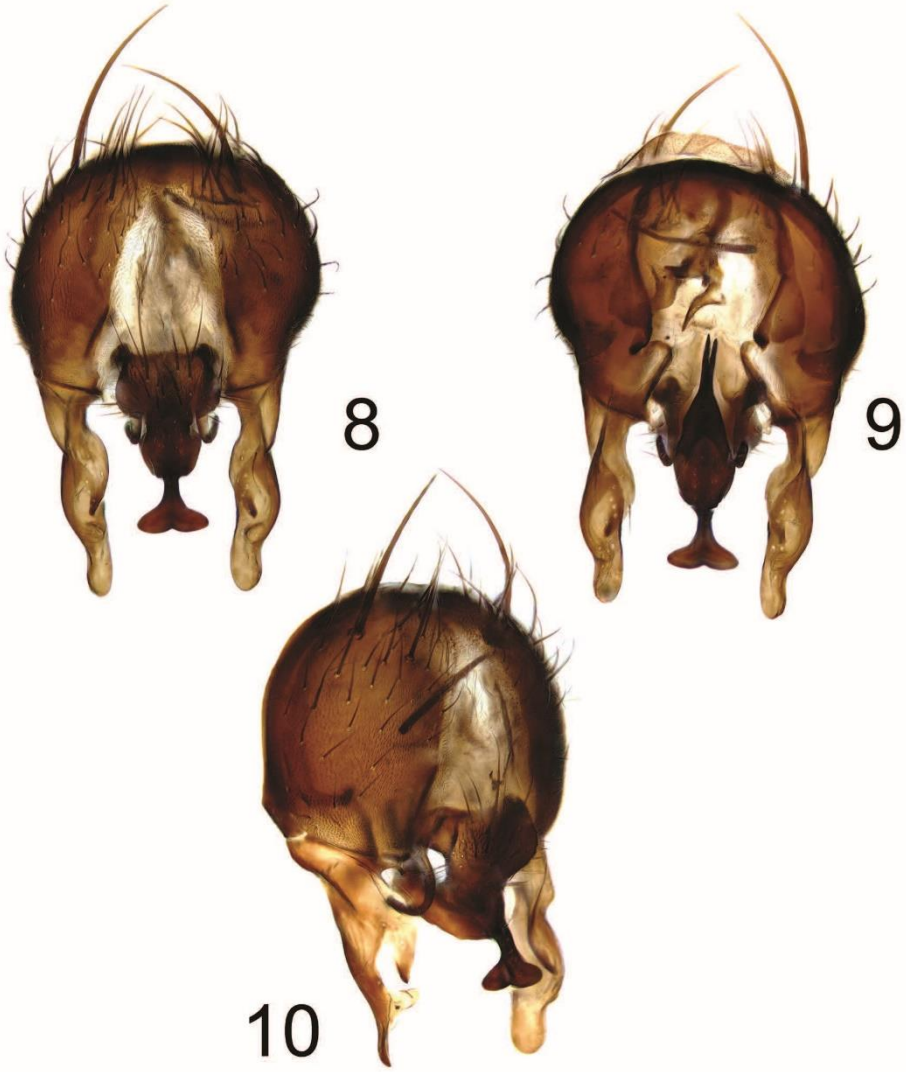
Fannia nidica Collin, 1939 (Figs 4–7). Material examined (18♂): 2♂: Bohemia mer., Vráž nr. Písek, 400 m, nr. brook, 49°23'59"N, 14°7'58"E, 10.v.-4.vi.2011; 1♂: same locality, 2.iv.-10.v.2011; 1♂: same locality, 30.iv.-6.vi.2012; 1♂: Moravia, Jihlava-Pávov, wetland nr. pond, 495 m, 49°26'26"N, 15°35'44"E, 16.iv.-3.v.2009; 1♂: Bílina – Vršíček, 50°33'12"N, 13°49'57"E, 410 m, 30.iv.-13.v.1998, - all M. Barták (- all MT, CULSP); 10♂: Bohemia b., Frýdlantská pahorkatina Hills, Poustecká obora nr. Poustka, 50°57'34"N, 15°3'51"E, 27.iv.-16.v.2012, MT, J. Preisler & P. Voníčka; 1♂: Bohemia b., Frýdlantská pahorkatina Hills, Černousy-V Poli nr. Dubový rybník, 50°59'46"N, 15°2'49"E, 16.v.-12.vi.2012, MT, J. Preisler & P. Voníčka; 1♂: Bohemia b., Jizerské hory Mts, Šolcův rybník, env. Raspenava, 350 m, 50°52'49"N, 15°6'51"E, MT, 11.-26.v.2011, J. Preisler & P. Voníčka (- all NBML). Very rare species, known only from England, Denmark and the Czech Republic (Sadská, Vršíček in NW Bohemia, and Podyjí NP – Gregor, Rozkošný, Barták & Kubík 2005). *Fannia nidica* has been erroneously placed in the key by Rozkošný et al. (1997) under couplet 31. However; it has usually 2 anterodorsal and 2 posterodosal setae on mid tibia (occasionally only 1 may be present on either side), which in fact leads the species to section 22. Moreover, *F. nidica* has relatively long (even if sparse) ommatrichia which may erroneously lead it to *F. hirticeps* in keys. However, the latter species has much narrower cercal plate, dark tip of halter and much shorter and broader midbasitarsal crest.

Fannia norvegica Ringdahl, 1934 (Figs 8–10). Material examined (6♂): 1♂: Bohemia occ., Duchcov, 2 km E, willow shrubs, 50°37'N, 13°43'E, 240 m, 8.vii.1992, M. Barták; 2♂: Bohemia occ., Bílina, Choumek hill, 50°32'38"N, 13°51'32"E, 480



Figures 4–7. *Fannia nidica* Collin, 1939, hypopygium: **4** surstylus, standardized view (appearing broadest) **5** dorsal view **6** bacilliform scerite **7** ventral view.

m, 24.vii.-24.viii.1998, MT, M. Barták; 1♂: Vráž nr. Písek, 400 m, 49°24'12"N, 14°7'3"E, 12.vi.-10.ix.2015, pyramidal trap with decaying wood, M. Barták (- all CULSP); 1♂: Mile 315 Alaska Richard. Hwy, 8.vi.1951 W. R. M. Mason (CNC – paratype of *F. lucida* Chillcott, 1961); 1♂: Wychwood Forest Oxon 1.vii.72, E. A. Fonseca, Pres. by E. C. M. Assis Fonseca BMNH 1988-212 (NHM). Broadly distributed, but uncommon species. Known from Norway, Spain, North Africa, G. Britain, Denmark, Greek, Switzerland and Japan. From the Czech Republic published from Bílina and Duchcov environs by Gregor and Barták (2001). *Fannia norvegica* was keyed by Wang et al. (2009) and they found it the closest to *F. lucida* Chillcott. It aroused our interest in the study of differences between these two species; moreover, cercal plate of our specimens seemed to be more similar to *F. lucida* (figured by Chillcott, 1961, fig. 74) than to *F. norvegica* (figured by d'Assis-Fonseca, 1968, fig. 37) especially by short "stem" before knob-like tip. Wang et al. (2009) stated differences between them as follows: "mid tibia with 2 ad; male cerci broad in distal half from ventral view, only apex slender" - *F. lucida*, and: „mid tibia with 3 ad; male cerci distinctly slender in distal half from ventral view, slightly broadened at apex" - *F. norvegica*. Ringdahl (1934) in original description also described mid tibia with 3 anterodorsals; however, Nishida (2003) redescribing *F. norvegica* stated: "mid tibia with 2 ad and 1-2 pd setae". In the original description of *F. lucida* (Chillcott 1961), there is stated: „separable... from *norvegica* by the



Figures 8–10. *Fannia norvegica* Ringdahl, 1934, hypopygium: **8** dorsal view **9** ventral view **10** oblique view.

fewer tibial bristles", but, their number is specified only in case of mid tibia: "two ads, two pds". Collin (1958, Fig. 1a) noticed „projection X" as a feature differing it from near *F. carbonaria* (beside presence of long posteroventrals on hind femur). To elucidate status of *F. lucida*, we borrowed one paratype specimen from CNC and found both species to be identical. The number of tibial setae is summarised in the Table 1. It seems clear that there is no difference between *F. lucida* and *F. norvegica* tibial setation.

Table 1. Setation of mid and hind tibia in all available specimens of *F. norvegica*.

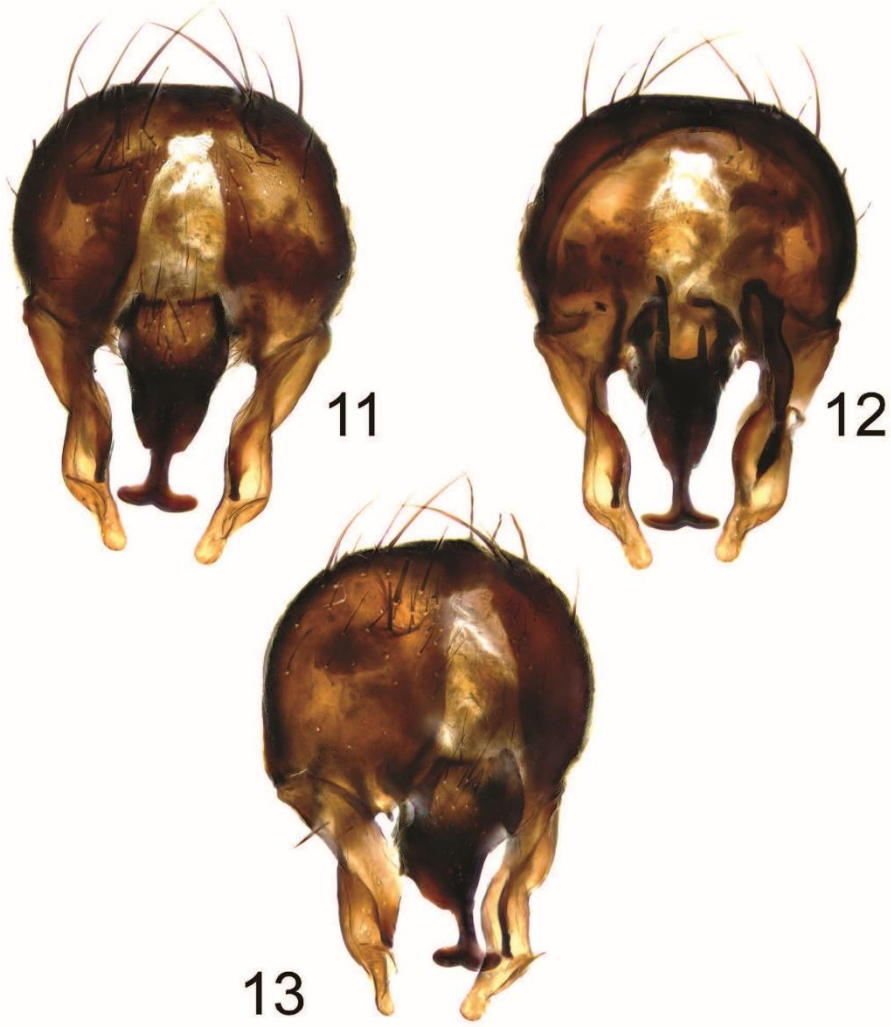
	Mid tibia	Hind tibia
Paratype of *F. lucida*	2 ad (dorsal one short), 2 pd (dorsal shorter)	3 av (ventral very short), 2 ad, 2 d
Duchcov specimen	2 ad, 2 pd (all nearly equal in size)	3 av, 3–4 ad, 2 d
Vráž specimen	2 ad, 1–2 pd	2 av, 3 ad, 2 d
Chloumek specimen 1	2 ad (dorsal one shorter), 2 pd (nearly equal in size)	1–2 av, 2 ad, 2 d
Chloumek specimen 2	2 ad (dorsal one shorter), 2 pd (nearly equal in size)	1-2 av, 2–3 ad, 2 d
Wychwood specimen	2 ad, 2 pd	2 av, 2 ad, 2 d

Studying genitalia of both species we found them identical including basal outgrowth of surstyli (Collin´s 1958 "projection X" - Fig. 10), simply bent bacilliform sclerites and forked (V-shaped) tip of ventral part of cercal plate (Fig. 9). Thus, *F. lucida* Chillcott, 1961 is considered here junior synonym of *F. norvegica* Ringdahl, 1934. Interestingly, another species very similar to *F. norvegica* is *F. pseudonorvegica* d´Assis-Fonseca, 1966. The latter species differs only in details from *F. norvegica*, beside small crest on the base of mid basitarsus, basal process of surstyli seems to be larger (Fig. 13), apical broadening of cercal plate narrower (more linear than heart-shaped), and ventral process of cercal plate ends in two basally separated (U-shaped) processes (Fig. 12).

Fannia slovaca Gregor & Rozkošný, 2005. Material examined: 1♂, Bohemia occ., Bílina, Chloumek, hilltop steppe, 480 m, 50°32'38"N, 13°51'32"E, 25.vi.-24.vii.1998, MT, M. Barták (CULSP). Species recognized only recently, so its distribution is only poorly known, so far found only in Slovak Republic and Finland (Kahanpää and Haarto 2014). First record for the Czech Republic.

Fannia verrallii (Stein, 1895). Material examined (3♂): 1♂: Bohemia b., Krkonoše Mts, Labský důl nr. Labe river, 1040 m, 50°45'48"N, 15°33'05"E, 21.-28.vi.2006, MT, J. Vaněk; 1♂: Bohemia occ., Šumava Mts, Rokytecká slať peatbog, 1100 m, 49°0'59"N, 13°25'5"E, 20.vii.-24.ix.1999, MT, M. Barták & Š. Kubík (- all CULSP); 1♂: Bohemia b., Jizerské hory Mts, Jizerka, 20.vi.2008, SW, J. Preisler (NBML). A rarely collected Holarctic species known in Europe only from G. Britain, Germany, Norway, Finland, Sweden, and the Czech Republic (Pont 2007). From Bohemia published by Gregor et al. (2003). Listed in Red list as vulnerable species in the Czech Republic (Gregor, Rozkošný and Barták 2005). Confirmed occurrence of this species in the Czech Republic and further records from Bohemia.

Fannia vespertilionis Ringdahl, 1934. 1♂: Bohemia c., Tiché údolí, Roztocký háj nr. Roztoky, 50°8'47.5"N, 14°23'10.1"E, 20.iv.-20.v.2009, beer trap, J. Preisler (NBML). Temperate European species. From the Czech Republic previously known only from Pálava BR (Gregor and Rozkošný 1999). Listed in Red list as vulnerable species in the Czech Republic (Gregor, Rozkošný and Barták 2005). First record from Bohemia.



Figures 11–13. *Fannia pseudonorvegica* d´Assis-Fonseca, 1966, hypopygium: **11** dorsal view **12** ventral view **13** oblique view.

An updated key to males of European species of *Fannia*

(The male of *F. latifrontalis* Hennig is not known; all species included in Fauna Europea are keyed as well as all species described more recently.)

1 Abdomen club-like, broadest just beyond middle (Rozkošný et al. 1997, Fig. 4q) **2**

– Abdomen normal, broadest in anterior half or at middle (Rozkošný et al. 1997, fig. 4r–t) **3**

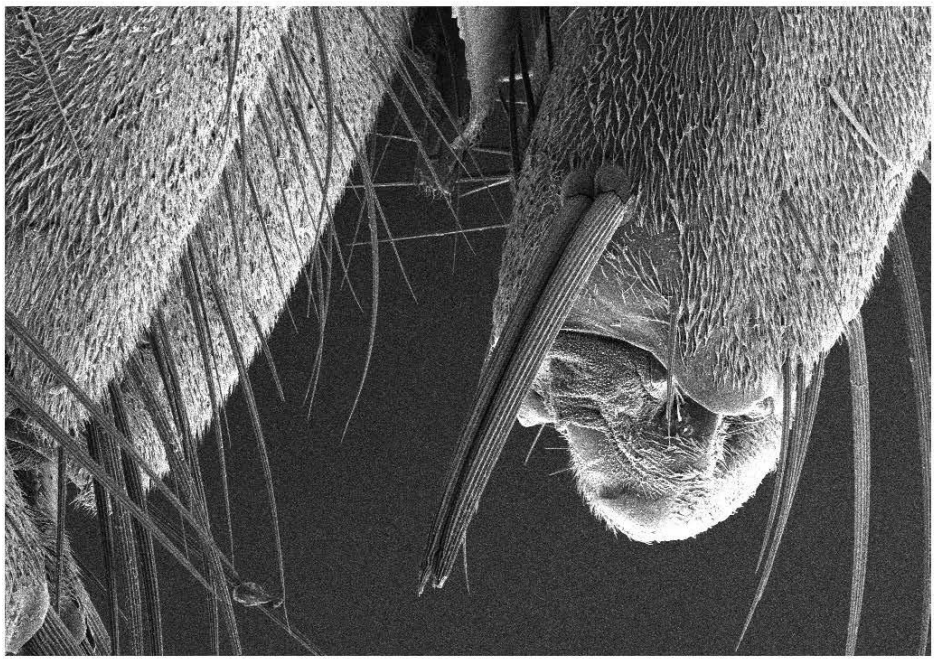


Figure 14. *Fannia manicata* (Meigen, 1826), two grooved spines standing side by side on fore coxa.

2 (1) Lower margin of face distinctly produced, theca of proboscis longer than half length of fore tibia (Rozkošný et al. 1997, fig. 3c); abdomen entirely black; ventral parts of tergites 4 and 5 with long crossing setae (Rozkošný et al. 1997, fig. 4q) (terminalia: Rozkošný et al. 1997, fig. 11d) ..... ***F. mollissima* (Haliday)**

– Lower margin of face barely produced, theca of proboscis much shorter; abdomen with a yellow pattern in basal half; ventral part of tergites without crossing setae (terminalia: Rozkošný et al. 1997, fig. 16e) ..... ***F. subpellucens* (Zetterstedt)**

3 (1) Mid coxa with 1–3 strong hook-like setae; hind coxa with 1 or more setae on posterior inner margin (Rozkošný et al. 1997, fig. 4o); presutural acrostichal setulae triseriate **4**

– Mid coxa without strong hook-like setae..... **12**

4 (3) Mid coxa with 2–3 hook-like setae (Rozkošný et al. 1997, fig. 4o); mid tibia with a shining black inner projection (Rozkošný et al. 1997, fig. 4e) (terminalia: Rozkošný et al. 1997, fig. 13e) ..... ***F. scalaris* (Fabricius)**

– Mid coxa with 1 hook-like seta; mid tibia without inner projection..... **5**

5 (4) Fore tibia with a dense brush of flattened setae at apex laterally (Rozkošný et al. 1997, fig. 4m); fore coxa on lower inner margin with two grooved spines standing side by side (Fig. 14)..... **6**

– Fore tibia without a brush of flattened setae; fore coxa without spines on lower inner margin 7

6 (5) Hind femur with strong anteroventral setae along almost whole length; hind tibia with a row of unequal posteroventral setae in apical 2/3; mid tibia remarkably dilated in apical half (terminalia: Rozkošný et al. 1997, fig. 10h) .... ***F. manicata*** **(Meigen)**

– Hind femur only with 2–3 anteroventral setae before apex; hind tibia without posteroventral setae; mid tibia only slightly dilated in apical half (terminalia: Rozkošný et al. 1997, fig. 11e) ..... ***F. monilis*** **(Haliday)**

7 (5) Katepisternum with a straight spine on ventral side; at least hind tibia pale, yellow to reddish brown **8**

– Katepisternum without straight spine on ventral side; all tibiae predominantly black **10**

8 (7) Mid and hind femora yellow; hind tibia with a row of long fine anteroventral setae in apical 2/3, its ventral and posteroventral surface covered with dense short setae (terminalia: Rozkošný et al. 1997, fig. 10g) ***F. lustrator*** **(Harris)**

– Mid and hind femora black; anteroventrals and ventral pubescence on hind tibia less conspicuous **9**

9 (8) Abdomen with a narrow undusted median stripe in posterior view; mid tibia only slightly dilated in apical half; hind tibia long and densely haired on ventral and posteroventral surfaces (terminalia: Rozkošný et al. 1997, fig. 8f) .... ***F. fuscula*** **(Fallén)**

– Abdomen with a median row of trapezoid dark spots dilated towards hind margin of tergites; mid tibia remarkably dilated in apical half; hind tibia without long fine hairs (terminalia: Rozkošný et al. 1997, fig. 15d) .... ***F. vesparia*** **(Meade)**

10 (7) Hind femur with only 4 strong anteroventral setae before apex; hind tibia with complete rows of long and fine anteroventral and anterodorsal setae; lower calypter brown, with almost black margin and fringe (terminalia:Rozkošný et al. 1997, fig. 11a) ..... ***F. melania*** **(Dufour)**

– Hind femur with a complete row of about 12 anteroventral setae; hind tibia at most with 8 anterodorsal and 6 anteroventral setae; lower calypter white, with yellowish margin and fringe **11**

11 (10) Palpi as broad as half width of flagellomere; several rows of setulae behind postocular setulae; fore tibia with a distinct anterodorsal seta (terminalia: Rozkošný et al. 1997, fig. 7a) ..... ***F. atripes*** **(Stein)**

– Palpi much less than half width of flagellomere; only one row of setulae behind postocular row; fore tibia without anterodorsal seta (terminalia: Rozkošný et al. 1997, fig.14c) ..... ***F. subatripes*** **d'Assis-Fonseca**

12 (3) Mid coxa with 2 short peg-like setae on outer side (Rozkošný et al. 1997, fig. 9d) (Finland) ..... ***F. rabdionata*** **Karl**

– Mid coxa without strong setae on outer side **13**

13 (12) Abdomen with a brown pattern on abdominal tergites 3 and 4 consisting of 2 pairs of round spots and a median vitta (cf. Rozkošný et al. 1997, fig. 4u) **14**
– Abdomen without a pattern of paired spots **15**
14 (13) Hind tibia with 1 anteroventral and 0 posteroventral seta; hind femur without a preapical ventral swelling, with the anteroventral setae only slightly longer than femoral depth and not curled at tips (terminalia: Rozkošný et al. 1997, fig. 10d) ..... ***F. leucosticta*** **(Meigen)**
– Hind tibia with numerous anteroventral and posteroventral setae; hind femur with a preapical ventral swelling bearing a number of long fine anteroventral setae that are longer than femoral depth and are curled at tips (terminalia: Rozkošný et al.1997, fig. 14h) ..... ***F. pusio*** **(Wiedemann)**
15 (13) Mid basal tarsomere with a crest (small spine- or toothlike process at extreme base ventrally) (Rozkošný et al. 1997, fig. 3p–r, 4j, 9e–f); inner posterior margin of hind coxa always bare **16**
– Mid basal tarsomere without crest; inner posterior margin of hind coxa with setae or bare **33**
16 (15) Fore basal tarsomere with brush-like hairs ventrally; cercal plate with long setae (Rozkošný et al.1997, fig. 7b) ..... ***F. barbata*** **(Stein)**
– Fore basal tarsomere without conspicuous ventral hairs; cercal plate with normal setae **17**
17 (16) Eyes haired, hairs at least as long as diameter of anterior ocellus..... **18**
– Eyes bare or with only very short and sparse hairs **19**
18 (17) Mid tibia with 2 anterodorsal and 2–3 posterodorsal setae; hind femur with dense hairlike antero- and posteroventral setae; hind tibia with a normal preapical dorsal seta (terminalia: Rozkošný et al. 1997, fig. 9a) .... ***F. hirticeps*** **(Stein)**
– Mid tibia only with 1 antero- and 1 posterodorsal seta; hind femur with 3–6 anteroventral and without posteroventral setae; hind tibia without dorsal preapical seta (terminalia: Rozkošný et al. 1997, fig. 12b) (Great Britain)..... ..... ***F. novalis*** **Pont**
19 (17) Mid tibia with a remarkable tubercle in basal half; body densely grey dusted (terminalia: Rozkošný et al. 1997, fig. 10a)..... ***F. krimensis*** **Ringdahl**
– Mid tibia without a tubercle in basal half, at most slightly swollen; body less dusted **20**
20 (19) Mid tibia with 2–3 anterodorsal and 2 posterodorsal setae; hind femur with 3–4 anteroventral setae at apex..... **21**
– Mid tibia with 1 antero- and 1 posterodorsal seta; hind femur at most with 2 anteroventral setae at apex..... **24**
21 (20) Hind tibia clothed with long and dense ventral hairs and with several fine curled setae at apex (Rozkošný et al. 1997, fig. 4k; terminalia: Rozkošný et al. 1997, fig. 6f)..... ***F. armata*** **(Meigen)**
– Hind tibia without long hairs and curled setae..... **22**

22 (21) Cercal plate narrowed apically (terminalia: Figs 4–7); hind tibia with one anteroventral and one anterodorsal seta; midbasitarsal crest very long (as long as or longer than diameter of mid tibia) and very narrow (only slightly broader than preapical setae); one preapical anterior and one preapical posterior seta on mid tibia ..... ***F. nidica*** **Collin**

– Cercal plate broadened apically; remaining characters different **23**

23 (22) Hind tibia with one anteroventral and one anterodorsal seta; postocular setulae uniserial (terminalia: Rozkošný et al. 1997, fig. 8c) ..... ***F. cothurnata*** **(Loew)**

– Hind tibia with 1–3 anteroventral and 2–3 anterodorsal setae; postocular setulae biserial (terminalia: figs 11–13) ..... ***F. pseudonorvegica*** **d'Assis-Fonseca**

24 (20) Hind femur without distinct anteroventral setae (terminalia: Rozkošný et al. 1997, fig. 13d); lower calypter strip-like ..... ***F. rondanii*** **(Strobl)**

– Hind femur with at least 1 strong anteroventral seta; lower calypter developed, lobe-like **25**

25 (24) Hind femur without posteroventral setae in apical half (terminalia: Rozkošný et al. 1997, fig. 10e) ..... ***F. limbata*** **(Tiensuu)**

– Hind femur with a row of posteroventral setae in apical half **26**

26 (25) Hind femur with 2–5 anteroventral setae before apex (terminalia: Rozkošný et al. 1997, fig. 13c) ..... ***F. ringdahlana*** **Collin**

– Hind femur with only 1 anteroventral seta before apex **27**

27 (26) Fore tibia with a row of elongate posteroventral hairs; cercal plate broad, deeply constricted before middle (Rozkošný et al. 1997, fig. 14a) ..... ***F. spathiophora*** **Malloch**

– Fore tibia without elongate posteroventral hairs; cercal plate without constriction before middle **28**

28 (27) Hind femur with 3–6 posteroventral setae **29**

– Hind femur with 7–14 posteroventral setae **30**

29 (28) Presutural acrostichal setulae triserial; ventral crest on mid basal tarsomere weak (Rozkošný et al. 1997, fig. 9e) (terminalia: Rozkošný et al. 1997, fig. 16a) ..... ***F. aethiops*** **Malloch**

– Presutural acrostichal setulae biserial; ventral crest on mid basal tarsomere well developed (Rozkošný et al. 1997, fig. 9f) (terminalia: Rozkošný et al. 1997, fig. 16d) (N Europe) ..... ***F. stigi*** **Rognes**

30 (28) Postocular setulae biserial; acrostichal setulae mainly triserial; mid tibia strongly flattened, with a posteroventral ridge in apical third (terminalia: Rozkošný et al. 1997, fig. 16c) ..... ***F. bigelowi*** **Chillcott**

– Postocular setulae uniserial; acrostichal setulae mainly biserial; mid tibia not strongly flattened **31**

31 (30) Scutum not pale dusted in front of scutellum, completely black; bacilliform process simply bent ventrally, long (Gregor and Rozkošný 2005, fig. 11) ..... ***F. umbratica*** **Collin**

– Scutum pale dusted in front of scutellum; bacilliform process spiralled, long or short **32**

32 (31) Ten to fifteen strong posteroventrals on hind femur (Gregor and Rozkošný 2005, fig. 9); bacilliform process short (Gregor and Rozkošný 2005, fig. 8)..... ***F. umbrosa* (Stein)**

– Five strong posteroventrals on hind femur (Gregor and Rozkošný 2005, fig. 10); bacilliform process long (Gregor and Rozkošný 2005, fig. 7)..... ***F. slovaca* Gregor & Rozkošný**

33 (15) Mid and hind femora yellow; abdomen with extensive yellow pattern or entirely reddish yellow..... **34**

– Mid and hind femora predominantly black; abdomen black, rarely with extensive yellow pattern **36**

34 (33) Inner posterior margin of hind coxa with 1 or more setae; abdomen including genitalia entirely reddish yellow (terminalia: Rozkošný et al. 1997, fig. 15e)..... ***F. vespertilionis* Ringdahl**

– Inner posterior margin of hind coxa bare; abdomen dark with yellow pattern..... **35**

35 (34) Mid tibia with a remarkable tubercle in middle (Rozkošný et al. 1997, fig. 4a); hind tibia at apex and hind basal tarsomere long haired ventrally; lower calypter not projecting (terminalia: Rozkošný et al. 1997, fig. 12c)..... ***F. ornata* (Meigen)**

– Mid tibia without median tubercle; hind leg without remarkable pubescence on tibia and basal tarsomere; lower calypter distinctly projecting (terminalia: Rozkošný et al. 1997, fig. 12h) ..... ***F. posticata* (Meigen)**

36 (33) Mid femur with a group of spine-like setae in middle (cf. Rozkošný et al. 1997, fig. 4a); hind tibia with only 1 dorsal seta, the preapical one absent (terminalia: Rozkošný et al. 1997, fig. 13h) ..... ***F. sociella* (Zetterstedt)**

– Mid femur without spine-like setae in middle; hind tibia always with 2 dorsal setae, median and preapical..... **37**

37 (36) Apex of abdomen globular; sternite 5 shining black, projecting downwards (terminalia: Rozkošný et al. 1997, fig. 8g, as *F. glaucescens*)..... ***F. lucidula* (Zetterstedt)**

– Apex of abdomen not globular; sternite 5 dull and adpressed..... **38**

38 (37) Inner posterior margin of hind coxa with setae (Rozkošný et al. 1997, fig. 4f)..... **39**

– Inner posterior margin of hind coxa bare..... **62**

39 (38) Mid tibia with a conspicuous tubercle on inner surface (Rozkošný et al. 1997, fig. 4d)..... **40**

– Mid tibia without tubercle on inner surface..... **41**

40 (39) Tubercle on mid tibia below middle; presutural acrostichal setulae in 3–4 rows; 1 long and fine prealar seta; hind tibia with about 8 anteroventral setae (terminalia: Rozkošný et al. 1997, fig. 7h) ..... ***F. coracina* (Loew)**

– Tubercle on mid tibia above middle (Rozkošný et al. 1997, fig. 4d); presutural acrostichal setulae in 2 rows; 2 short prealar setae; hind tibia only with

1 anteroventral seta (terminalia: Rozkošný et al. 1997, fig. 14f) ..... ***F. tuberculata* (Zetterstedt)**

41 (39) Mid tibia along whole length with dense, short, uniform and erect ventral pubescence, about half as long as greatest diameter of tibia or shorter (Rozkošný et al. 1997, fig. 4b); presutural acrostichal setulae triserial **42**

– Mid tibia ventrally with sparser, not uniform and especially in apical half usually much longer hairs (Rozkošný et al. 1997, fig. 4c); presutural acrostichal setulae mostly biserial **55**

42 (41) Abdomen yellowish at least at base **43**

– Abdomen entirely black **47**

43 (42) Fronto-orbital plates separated by a narrow frontal vitta; mesonotum yellowish grey dusted, without longitudinal brown stripes; tibiae broadly yellow at bases (terminalia: Rozkošný et al. 1997, fig. 9b) ..... ***F. hirundinis* Ringdahl**

– Fronto-orbital plates touching at least in a short distance; mesonotum usually with conspicuous longitudinal stripes; at most fore tibia yellowish at base ... **44**

44 (43) Abdominal segments 2 and 3 predominantly yellow; black median vitta narrow, not dilated at posterior margin of tergites; scutum with 3 brown stripes (terminalia: Rozkošný et al. 1997, fig. 14b) ..... ***F. speciosa* (Villeneuve)**

– Abdominal segments 2 and 3 only with oval lateral yellow spots, black median vitta dilated towards posterior margin of tergites (Rozkošný et al. 1997, fig. 4r); scutum at most with 1 brown stripe **45**

45 (44) Several short setae distinct above anterodorsal seta on hind tibia; scutum with a median matt brown stripe (terminalia: Rozkošný et al. 1997, fig. 7c) ..... ***F. canicularis* (Linnaeus)**

– Without short setae above anterodorsal seta on hind tibia; scutum without median stripe **46**

46 (45) Proepisternal depression bare; hind femur shortly and densely haired on posteroventral surface; mid femur with short and dense antero- and posteroventral setae; prealar midway between suture and supra-alar seta (terminalia: Rozkošný et al. 1997, fig. 7f) ..... ***F. clara* Collin**

– Proepisternal depression with several small setulae; hind femur only with short and sparse fine hairs on posteroventral surface; setae on mid femur long and sparse; prealar closer to suture (terminalia: Rozkošný et al. 1997, fig. 8d) ..... ***F. difficilis* (Stein)**

47 (42) Hooked aedeagus present and usually exposed (Rudzinski 2003, fig. 2); surstyli broad and triangular in ventral view (Rudzinski 2003, fig. 4); proepimeral seta with two or more adjacent setulae; proepisternal depression without setae; hind tibia with 1–2 anteroventrals and no posteroventral ..... ***F. conspecta* Rudzinski**

– Aedeagus membranose; main process of surstyli narrow and parallel-sided; remaining characters different **48**

48 (47) Palpi dilated and flattened, almost as broad as antennal flagellomere (Rozkošný et al. 1997, fig. 3g); mid femur with several rows of strong setae

on posteroventral surface (terminalia: Rozkošný et al. 1997, fig. 10b)..... ..... ***F. latipalpis* (Stein)**

– Palpi not dilated and flattened; mid femur with uniserial (or exceptionally with 2–3 rows of) posteroventral setae **49**

49 (48) Distance between eye margins about twice as broad as antennal flagellomere; hind tibia with strong anterodorsal and dorsal setae at about same level (terminalia: Rozkošný et al. 1997, fig. 9c) ..... ***F. brinae* Albuquerque**

– Distance between eye margins much narrower; anterodorsal and dorsal setae on hind tibia inserted at different levels **50**

50 (49) Scutum with 2 longitudinal brown stripes; postocular setulae biserial; hind tibia with 5–7 posteroventral setae (terminalia: Rozkošný et al. 1997, fig. 9i)... ..... ***F. incisurata* (Zetterstedt)**

– Scutum with 1 or 3 longitudinal brown stripes or completely black; postocular setulae uniserial; hind tibia without posteroventral setae **51**

51 (50) Proepimeral seta surrounded by several setulae **52**

– Proepimeral seta with only 1 adjacent setula **53**

52 (51) Proepisternal depression with a few setulae; hind tibia with 1–2 anteroventral setae; hind femur with short posteroventral setae which are not as long as femoral depth (terminalia: Rozkošný et al. 1997, fig. 14g)..... ***F. monticola* Pont**

– Proepisternal depression bare; hind tibia with 2–5 anteroventral setae; hind femur with posteroventral setae that are much longer than femoral depth (terminalia: Rozkošný et al. 1997, fig. 6d) ..... ***F. aequilineata* Ringdahl**

53 (51) Squamae with brown margin; mesoscutum deep black; abdomen with bluish shine (Canary Islands)..... ***F. pubescens* Stein**

– Squamae without brown margin; mesoscutum light grey; abdomen without bluish shine.....**54**

54 (53) Hind tibia with 2 equally strong anteroventral setae; scutum with a median brown longitudinal stripe (dark form; see 44) ..... ***F. canicularis* (Linnaeus)**

– Hind tibia usually with 1 anteroventral seta; if 2 developed, then upper obviously shorter; scutum brownish black, without median stripe (terminalia: Rozkošný et al. 1997, fig. 14d) ..... ***F. subpubescens* Collin**

55 (41) Mid tibia with 2 or more antero- and posterodorsal setae (Rozkošný et al. 1997, fig. 4c) **56**

– Mid tibia only with 1 antero- and 1 posterodorsal seta **59**

56 (55) Hind femur in apical third with a tubercle bearing 12–15 posteroventral setae (terminalia: Rozkošný et al. 1997, fig. 16b) ..... ***F. lugubrina* (Zetterstedt)**

– Hind femur in apical third without tubercle **57**

57 (56) Ventral hairs on mid tibia not longer than greatest diameter of tibia; palpi shorter than half length of theca (Rozkošný et al. 1997, fig. 3e) (terminalia: Rozkošný et al. 1997, fig. 11c)..... ***F. minutipalpis* (Stein)**

– At least some ventral hairs on mid tibia longer than greatest diameter of tibia (Rozkošný et al. 1997, fig. 4c); palpi longer than half length of theca (Rozkošný et al. 1997, fig. 3d) **58**

58 (57) Hind tibia with 3–4 anteroventral setae; longest ventral hairs on mid tibia about 1.5 times longer than greatest diameter of tibia (fig 4c) (terminalia: Rozkošný et al. 1997, fig. 12f) ..... ***F. polychaeta* (Stein)**

– Hind tibia with only 1–2 anteroventrals; ventral hairs on mid tibia shorter though overreaching diameter of tibia (terminalia: Rozkošný et al. 1997, fig. 11h) ..... ***F. pauli* Pont**

59 (55) Prealar seta completely absent; presutural acrostichal setulae always biserial (terminalia: Rozkošný et al. 1997, fig. 5a–e) ..... ***F. genualis* (Stein)**

– One or two prealar setae present; presutural acrostichal setulae in 2 or 3 rows **60**

60 (59) Hind tibia without posteroventral setae; abdomen with a narrow median vitta which may be absent on tergite 5 (terminalia: Rozkošný et al. 1997, fig. 7g) ..... ***F. collini* d' Assis-Fonseca**

– Hind tibia with a distinct row of posteroventral setae; median spots on abdomen remarkably dilated towards posterior margin of tergites **61**

61 (60) Posteroventral setae on hind tibiae longer than anterodorsal setae (Rozkošný et al. 1997, fig. 4l); hind femur with a complete row of anteroventral setae, distal 4–5 of them stronger (terminalia: Rozkošný et al. 1997, fig. 9h) ..... ***F. immutica* Collin**

– Posteroventral and anterodorsal setae on hind tibia of the same length; hind femur with only 2 anteroventral setae (terminalia: Rozkošný et al. 1997, Fig. 10c) ..... ***F. lepida* (Wiedemann)**

62 (38) Upper half of parafacials with a row of short setulae (terminalia: Rozkošný et al. 1997, fig. 10f) ..... ***F. lineata* (Stein)**

– Parafacials bare, rarely with a few isolated setulae **63**

63 (62) Lower calypter very narrow, strip-like, narrower than 1/3 of upper calypter (Rozkošný et al. 1997, fig. 3m–o) **64**

– Lower calypter rounded, broader than 1/2 of upper calypter (cf. Rozkošný et al. 1997, fig. 3k–l) **71**

64 (63) Mid and hind tibiae reddish brown to yellow **65**

– All tibiae mainly black **66**

65 (64) Thorax and abdomen mainly black; abdomen with a median row of subtriangular spots (terminalia: Rozkošný et al. 1997, fig. 12d) ..... ***F. pallitibia* (Rondani)**

– Thorax and abdomen densely grey dusted; abdomen with a narrow median vitta (terminalia: Rozkošný et al. 1997, fig. 13a) ..... ***F. pruinosa* (Meigen)**

66 (64) Hind femur with 3–4 posteroventral setae in apical half equalling greatest width of femur **67**

– Hind femur without elongate posteroventral setae **68**

67 (66) Presutural acrostichal setulae triserial; cercal plate tapered in distal part (rozkošný et al. 1997, Fig. 6e) ..... ***F. alpina* Pont**

– Presutural acrostichal setulae biserial; cercal plate distally T-shaped dilated (Rozkošný et al. 1997, fig. 7e) ..... ***F. carbonella* (Stein)**

68 (66) Parafacials indistinct in lateral view; cercal plate with two rounded processes (terminalia: Rozkošný et al. 1997, fig. 12e)..... ***F. parva* (Stein)**

– Parafacials distinct in lateral view; cercal plate flat and dilated, without two rounded processes.....**69**

69 (68) Upper postocular setulae uniserial and uniform in length; mostly only one strong prealar seta; abdomen with a narrow median stripe on tergites 4 and 5 (terminalia: Rozkošný et al. 1997, fig. 13g)..... ***F. similis* (Stein)**

– Upper postocular setulae biserial or at least alternating long and much shorter ones; usually two prealar setae; abdomen with a narrow median stripe on tergites 4 and 5 or with dark spots dilated posteriorly **70**

70 (69) Abdomen with a dark median stripe of uniform width; fore tibia yellowish basally (terminalia: Rozkošný et al. 1997, fig. 14e)....***F. subsimilis* Ringdahl**

– Abdomen with dark spots dilated towards posterior margin of tergites; fore tibia usually dark basally (terminalia: Rozkošný et al. 1997, fig. 13f) ....***F. serena* (Fallén)**

71 (63) Hind femur with a preapical tubercle bearing a cluster of dense setae (Rozkošný et al. 1997, fig. 4g, h) **72**

– Hind femur without setose tubercle.....**73**

72 (71) Hind femur strongly curved (Rozkošný et al. 1997, fig. 4g); abdomen yellowish at base (terminalia: Rozkošný et al. 1997, fig. 8e) ....***F. fasciculata* (Loew)**

– Hind femur not curved (Rozkošný et al. 1997, fig. 4h); abdomen entirely dark (terminalia: Rozkošný et al. 1997, fig. 11 b) .... ***F. metallipennis* (Zetterstedt)**

73 (71) Mid tibia with only 1 anterodorsal seta.....**74**

– Mid tibia at least with 2 anterodorsal setae.....**79**

74 (73) Hind tibia with 3–4 anterodorsal and at least 2 anteroventral setae.....**75**

– Hind tibia with only 1 anterodorsal and 1 anteroventral seta.....**76**

75 (74) Hind tibia with about 10 long and fine anteroventral and numerous hairlike ventral and posteroventral setae (terminalia: Rozkošný et al. 1997, fig. 16f) (Balearics, N Africa)..... ***F. tunisiae* Chillcott**

– Hind tibia only with 2 anteroventral and without elongate ventral and posteroventral setae (terminalia: Rozkošný et al. 1997, fig. 11g).....***F. nigra* Malloch**

76 (74) Abdomen yellowish at base; cercal plate about 5 times longer than broad (terminalia: Rozkošný et al. 1997, fig. 8h) .....***F. gotlandica* Ringdahl**

– Abdomen entirely black; cercal plate broader.....77

77 (76) Hind femur with fine, long and curled posteroventral setae in basal half (terminalia: Rozkošný et al. 1997, fig. 15c) ..... ***F. verrallii* (Stein)**

– Hind femur in basal half with posteroventrals at most half as long as depth of femur.....**78**

78 (77) Hind femur with one strong anteroventral seta (terminalia: Rozkošný et al. 1997, fig. 16a) (form without ventral crest on mid basal tarsomere; see 29).... ..... ***F. aethiops* Malloch**

– Hind femur with 5–11 anteroventral setae (terminalia: Rozkošný et al. 1997, fig. 12g) ..... ***F. postica* (Stein)**

79 (73) Hind femur with long setae on ventral and posterior surface subapically, the longest of these about as long as half length of hind tibia (terminalia: Rozkošný et al. 1997, fig. 6g) ..... ***F. atra* (Stein)**

– Hind femur without such long setae in apical half **80**

80 (79) Halter dark apically; cercal plate about as long as broad, with two short divergent apical processes (terminalia: Rozkošný et al. 1997, fig. 8b); upper postoculars long and unequal in length (alternating long and short setae) and partly biserial ..... ***F. corvina* (Verrall)**

– Halter clear yellow; cercal plate different; upper postoculars equally short and uniserial (except *F. carbonaria*) **81**

81 (80) Hind femur with complete row of long posteroventrals longer than depth of femur in apical part; surstylus with basal outgrowth (terminalia: Figs 8–10) ... . ***F. norvegica* Ringdahl**

– Hind femur without posteroventrals or at most with short posteroventrals on basal part; surstylus without basal outgrowth **82**

82 (81) Cercal plate with two bowed ribs but without apical projection (terminalia: Rozkošný et al. 1997, fig. 8a) ..... ***F. fuscitibia* Stein**

– Cercal plate with apical projection button-like broadened apically but without two bowed ribs (terminalia: Figs 1–3) ..... ***F. carbonaria* (Meigen)**

Checklist of Czech and Slovak species

The last checklist of Czech and Slovak Fanniidae (Gregor and Rozkošný et al. 1997, 2009) contains 66 species: 64 from the Czech Republic (60 from Bohemia and 60 from Moravia) and 50 from Slovakia. Recently, *F. conspecta* and *F. latifrontalis* were published from the Czech Republic (Grzywacz and Prado e Castro 2012 and Preisler et al. 2013, respectively), which, together with 5 species first recorded herewith raised the number of known Czech species to 71. Slovak species are less known, two species have been added to last checklist (*F. tuberculata* and *F. speciosa*: Straka 2011) and another is added herewith raising the total number of known Slovak species to 53. All species previously published from the Czech Republic but not present in CULSP or NBML but deposited in NMP or MMB were checked to avoid the inclusion of questionable species.

[Arranged according to tradition of Czech and Slovak checklists of Diptera: Ježek (ed.) (1987), Chvála (ed.) (1997), Jedlička et al. (eds) (2006, 2009)]. B = Bohemia, M = Moravia, SK = Slovakia. All additions to current checklist (Gregor and Rozkošný et al. 1997, 2009) are signed with *.

***Piezura* Rondani, 1866**

graminicola (Zetterstedt, 1846) (B, M), SK
pardalina Rondani, 1866 (B, M) SK

***Fannia* Robineau-Desvoidy, 1830**

aequilineata Ringdahl, 1945 (B, M), SK
armata (Meigen, 1826) (B, M), SK
alpina Pont, 1970 (B, M*), SK*
atra (Stein, 1895) (B, M), SK
atripes Stein, 1916 (B, M)
barbata (Stein, 1892) (B, M), SK
brinae Albuquerque, 1951 M*
canicularis (Linnaeus, 1761) (B, M), SK
carbonaria (Meigen, 1826) (B, M), SK
carbonella (Stein, 1895) (B, M), SK
clara Collin, 1939 (B, M)
collini d'Assis-Fonseca, 1966 (B*)
conspecta Rudzinski, 2003 (B*, M*)
coracina (Loew, 1873) (B, M), SK
corvina (Verrall, 1892) (B, M), SK
cothurnata (Loew, 1873) (B*, M), SK
difficilis (Stein, 1895) (B, M), SK
fasciculata (Loew, 1873) (M)
fuscitibia Stein, 1920 (B, M)
fuscula (Fallén, 1825) (B, M), SK
genualis (Stein, 1895) (B, M), SK
hirticeps (Stein, 1892) (B, M), SK
immutica Collin, 1939 (B, M), SK
incisurata (Zetterstet, 1838) (B, M), SK
krimensis Ringdahl, 1934 (M), SK
latifrontalis Hennig, 1955 (B*)
latipalpis (Stein, 1892) (B, M), SK
lepida (Wiedemann, 1817) (B, M), SK
leucosticta (Meigen, 1838) (B, M), SK
limbata (Tiensuu, 1938) (B, M*)
lineata (Stein, 1895) (B, M)
lucidula (Zetterstedt, 1860) (B, M), SK
lugubrina (Zetterstedt, 1838) (B*)
lustrator (Harris, 1780) (B, M), SK
manicata (Meigen, 1826) (B, M), SK
melania (Dufour, 1839) (B*), SK
metallipennis (Zetterstedt, 1838) (B, M), SK
minutipalpis (Stein, 1895) (B, M), SK
mollissima (Haliday in Westwood, 1840) (B, M), SK
monilis (Haliday, 1838) (B, M), SK
nidica Collin, 1939 (B, M)
nigra Malloch, 1910 (B, M)

norvegica Ringdahl, 1934 (B)
ornata (Meigen, 1826) (B, M), SK
pallitibia (Rondani, 1866) (B, M), SK
parva (Stein, 1895) (B, M), SK
pauli Pont in Rozkošný, Gregor & Pont, 1997 (B, M), SK
polychaeta (Stein, 1895) (B, M), SK
postica (Stein, 1895) (B, M), SK
posticata (Meigen, 1826) (B, M), SK
pruinosa (Meigen, 1826) (B, M), SK
pseudonorvegica d´Assis-Fonseca, 1966 (B)
ringdahlana Collin, 1939 (B, M), SK
rondanii (Strobl, 1893) (B, M), SK
scalaris (Fabricius, 1794) (B, M), SK
serena (Fallén, 1825) (B, M), SK
similis (Stein, 1895) (B, M), SK
slovaca Gregor & Rozkošný, 2005 (B*) SK
sociella (Zetterstedt, 1845) (B, M), SK
spathiophora Malloch, 1918 (B, M)
speciosa (Villeneuve, 1898) (B, M) SK*
subpubescens Collin, 1958 (B, M), SK
subsimilis Ringdahl, 1934 (B, M), SK
tuberculata (Zetterstedt, 1849) (B, M), SK*
umbratica Collin, 1939 (B, M), SK
umbrosa (Stein, 1895) (B, M), SK
verrallii (Stein, 1895) (B, M)
vesparia (Meade, 1891) (B, M), SK
vespertilionis Ringdahl, 1934 (B*, M)

Discussion

There are three important records of Central European Fanniidae that have mostly been overlooked because they were published in small local proceedings or journals:

Fannia speciosa: Eurasian species, recorded from Japan by Nishida (1976). In spite of being considered rare in central Europe (Rozkošný et al. 1997), we found surprisingly large numbers of specimens in Vráž near Písek (some 500 specimens, mostly females), especially from a pyramidal trap inserted above a large heap of decaying wood (see Preisler et al. 2013). From Slovakia reported only recently by Straka (2011).

Fannia latifrontalis: from the Czech Republic known only from a single female taken in Vráž near Písek (Preisler et al. 2013). For further comments about this seemingly very rare species see Kahanpää and Haarto (2014).

Fannia tuberculata: another rare species known previously from only two Czech Republic records: Mariánské Lázně and Lačnov near. Valašské Klobúky (see Rozkošný and Gregor 1988). From Slovak Republic reported by Straka (2011).

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SHORT RESEARCH AND DISCUSSION ARTICLE

Importance of fish gender as a factor in environmental monitoring of mercury

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Abstract Total mercury concentrations were determined in the gonadal tissues of 15 female and 10 male European perch (*Perca fluviatilis*) from one location of the stream "Jevanský potok" located about 30 km from Prague (Czech Republic). Tissue samples were frozen at −26 °C in polypropylene containers until further processing, which was carried out using an Advance Mercury Analyser (single purpose atomic absorption spectrometer). Mercury concentrations were present in all analysed gonad samples, and ranged from 2.3 to 12.7 μg/kg wet weight. However, we determined a mean Hg concentration (9.45 μg/kg) in male gonads that was 2.4 times greater than that of female gonads (3.9 μg/kg). This is an important finding when taking into account fish sex in environmental pollution monitoring (especially for mercury contamination).

Keywords Mercury · Accumulation · Gonad · Sex · *Perca fluviatilis*

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Introduction

Due to its toxicity and accumulation in organisms (particularly fish), mercury is an important pollutant of concern as well as one of the most widely studied. However, the factors that control bioconcentration and accumulation are not completely understood (Deng et al. 2013). Mercury enters the aquatic ecosystem in the form of inorganic mercury. Bacteria in water and sediments convert the inorganic mercury into highly toxic methylmercury (Gilmour and Henry 1991). The methylation of inorganic mercury in the aquatic environment and subsequent bioaccumulation and biomagnification of this compound are greater in animals found near the top of the food chain (i.e. predatory animals). Therefore, there are elevated levels of methylmercury in the tissues of predatory fish (Ryman et al. 2008). Relatively small concentrations of Hg (<160 μg/g wet weight as whole fish) can adversely affect the reproduction, development, growth, metabolism, behaviour and immune responses of fish (Rhea et al. 2013). Moreover, reports about intersex fish have been described by many authors (Hinck et al. 2009; Geraudie et al. 2011; Tetreault et al. 2011; Hou et al. 2011). The mechanism or mechanisms responsible for intersex is not known, but many factors including pollutants can influence sex differentiation in fish (Devlin and Nagahama 2002). During the period when gonads begin to evolve, exposure to mercury can affect their development. Mercury can be associated with endocrine disruption in fish (Hinck et al. 2009).

This study examined the inter-relations between the sex of free living fish (*Perca fluviatilis*) and mercury accumulation in their gonadal tissues.

Materials and methods

In March 2012, 25 specimens of European perch (*P. fluviatilis*) with mean body lengths of 23.0 cm (min 21.5–max 25.5) and

Fig. 1 Map with the stream locality "Jevanský potok" (GPS: 49057'47°N 14048'43 E) in Central Region and is situated 450 m from the nearest road

body weights of 175.0 g (min 164.5 max 202.5) were taken from one location of the Jevanský potok stream, (Fig. 1) located about 30 km from Prague (there were no official sources of mercury exposure), and frozen immediately. The mean body length (23 cm) and body weight (175 g) were the same for both male and female fish species. All of the specimens were taken from the same location of the Jevanský potok stream.

After the fish were brought to the laboratory, they were necropsied and the gonads were removed from the fish body. We estimated that the gonads had reached the mature stage. Unfortunately, we did not analyse the precise stage of maturation, but we assumed that the previtellogenic oocytes and oocytes in the final maturation stage were present in females, and that the testes in males were fully developed.

No helminth parasites were found in the fish intestines. Fish gonad tissue samples were taken with the aid of stainless steel scissors and forceps, which had been cleaned with redistilled water. Tissue samples were frozen at −26 °C in polypropylene containers until further processing, which was carried out using an Advance Mercury Analyser (AMA 254, Altec, Ltd., Czech Republic). AMA 254 is single purpose atomic absorption spectrometer, developer and produced in CR. It is used for assessment of total content of mercury in solid and also in liquid samples without previous amendments of samples. Values of mercury are presented on wet weight (w.w.) basis in milligrams per kilogram.

Basic descriptive statistics were computed. The normality of the data was tested separately using a Shapiro-Wilk test. When the assumptions of parametric statistics could be met, a nonparametric Mann Whitney *U* test was used for the evaluation the proposed hypothesis. Statistica ver. 10 (Statsoft 2011) was used for all computations and statistical analyses.

Results and discussion

Carnivorous fish species can be considered top consumers in aquatic ecosystems and represent a potential risk to other fish consumers, particularly humans. In a previous study (Jankovska et al. 2012; Table 1) from a monitored area near Prague (Jevanský potok), the muscle tissues of perch were found to contain an average of 11.54 μg of total Hg (100 g × 0.1,154 μg/g). The gonadal tissues accumulated ten times less

Table 1 Hg concentrations (mg/kg) in tissues of fish and parasite determined in previous study (Jankovska et al. 2012)

Fish number	Muscle (mg/kg)	Gonads (mg/kg)	*A. lucii* (mg/kg)	Number
2 F	0.1494	0.0099	0.0005	1
4 F	0.0903	0.0113	0.0045	3
5 F	0.0884	0.0091	0.0130	5
6 M	**0.1119**	**0.0231**	**0.0066**	1
7 F	0.1027	0.0106	0.0133	3
8 F	0.1106	0.0136	0.0174	3
10 F	0.1324	0.0126	0.0061	2
11 F	0.1311	0.0170	0.0130	5
13 F	0.1218	0.0136	0.0149	12
Mean	**0.1154**	**0.0134**	**0.0099**	
SD	**0.019**	**0.004**	**0.005**	

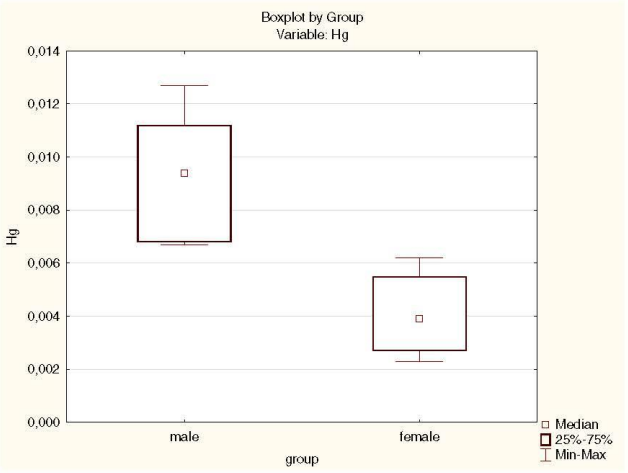

Fig. 2 Boxplot showing Hg concentrations (mg/kg) in gonads of male and female of perch (*P. fluviatilis*). The mean concentration is 0.00945 mg/kg (SD±0.0023) and 0.0039 mg/kg (SD±0.0014) for male and female, respectively

mercury (0.01 mg/kg) than did the muscle tissues (0.1 mg/kg) of perch (*Perca fluviatilis*). Similarly, Tóth et al. (2012) monitored Hg content in carp (*Cyprinus carpio*) in south-west Slovakia (near regional town Nitra) and determined 0.07 and 0.01 mg/kg in muscle and gonadal tissues, respectively. Unfortunately, Tóth et al. (2012) did not report whether the Hg concentrations were in male or female gonads. In a previous study (Jankovska et al. 2012), we found no differences Hg concentration/accumulation in muscle tissue of male and female fish (Table 1). However, this study did confirm Hg level differences between Hg in the gonadal tissues of male fish and those of female tissues (Fig. 2). Mercury was present in all analysed gonad samples and concentrations ranged from 2.3 to 12.7 μg/kg w.w. Mean (median)±SD Hg concentrations (in milligrams per kilogram w.w.) in male fish gonadal tissue were 9.45(9.44)±2.3 (min–max 6.7–12.7). Female mean (median)±SD were 3.9(3.9)±1.3 (min–max 2.3–6.2). Tolerable limit for Hg content in fish muscle (0.5 mg/kg w.w.=500 μg/kg w.w.) was not exceeded.

Table 2 Hg concentrations (mg/kg) in male and female gonadal tissues of perch

Fish sex	Hg
1. Male	0.0112
2. Male	0.0067
3. Male	0.0068
4. Male	0.0105
5. Male	0.0088
6. Male	0.0094
7. Male	0.0127
Male mean±SD	**0.00945±0.0023**
Male median±SD	**0.00944±0.0023**
Min–max	**0.0067–0.0127**
8. Female	0.0029
9. Female	0.0023
10. Female	0.0055
11. Female	0.0025
12. Female	0.0039
13. Female	0.0042
14. Female	0.0062
15. Female	0.0055
16. Female	0.0027
17. Female	0.0033
18. Female	0.0039
Female mean±SD	**0.0039±0.0014**
Female median±SD	**0.0039±0.0013**
Min–max	**0.0023–0.062**

Mercury can be biologically transformed into methylmercury, where it bioaccumulates up the food chain (Furl and Meredith 2011). One hypothesis asserts that mercury in the aquatic environment impacts the reproductive health of fish. Crump and Trudeau (2009) present the inhibitory effects of mercury on reproduction that occur at multiple sites within the reproductive axis, including the hypothalamus, pituitary and gonads. At the level of the pituitary gland, mercury exposure has inactivated and/or reduced the number of gonadotropin-secreting cells. Crump and Trudeau (2009) also examined the effect of mercury on the reproductive organs and demonstrated a range of effects, including reductions in gonad size, circulating reproductive steroids, gamete production and spawning success. There is increasing evidence that health effects of toxic metals differ in prevalence or are manifested differently in male and female animals (Vahter et al. 2007). In

this study, the mean Hg concentration in male perch gonads (9.45±2.2 μg/kg) was 2.4 times higher than that in the female gonads (3.9±1.4 μg/kg). There was statistically significant differences between Hg concentrations in male and female gonads (M-W U test, $p<0.05$). For details, see Fig. 2 and Table 2. Also, Brázová et al. (2012) reported the mean Hg concentration in male perch gonads (0.305 μg/g); it is 3.5 times higher than that in the female gonads (0.087 μg/g).

Mercury bioaccumulation in the gonads generally occurs as a result of the transportation of nutrients for maturation. These nutrients usually accumulate rapidly over the short time period of maturation and would be completely consumed after spawning is complete.

The amount of Hg transported in gonads along with nutrients may be high as the fish matures but may decrease dramatically as the gonads degenerate after sparing (Liu et al. 2013).

Our and Brázová et al. (2012) results suggest that male gonads (milt) accumulate higher levels of Hg than do female spawn. Higher mercury concentrations have also been observed in male seals by Marino et al. (2011), and in male red foxes by Lanocha et al. (2012). The reasons male gonads accumulate greater Hg concentrations than those of females are so far unknown. However, different compositions of male and female gonadal tissues may serve as one explanation/theory. Other reasons why male gonads accumulated higher Hg concentrations than did female gonads will be answered through further research.

The influence of species sex on the use of fish in environmental pollution monitoring studies (especially for mercury contamination) must be taken into account.

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Trichomonas spp. in Pigeons: Detection by OSOM Trichomonas Rapid Test

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Research Note—

Trichomonas spp. in Pigeons: Detection by OSOM Trichomonas Rapid Test

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SUMMARY. The efficacy of the OSOM Trichomonas Rapid Test (developed for rapid diagnosis of human *Trichomonas vaginalis*) in detection of *Trichomonas* spp. in pigeons (*Columba livia*) was investigated. Two oral cavity swabs were taken from 50 farm pigeons. Cultivation in Diamond Trichomonas medium was used as a reference method. According to a morphological determination, *Trichomonas gallinae* was the only protozoan found; however, no further molecular analysis was conducted. The OSOM Trichomonas test was positive in 39 oral swabs. In comparison with the cultivation method three samples were identified as false negative and one as false positive. Test specificity and sensitivity were established as 93% and 90%, respectively. Using Cohen's Kappa, the concordance between the two testing methods was found to be strong ($\kappa = 0.7506$, 95% CI = 0.5162–0.9850). The OSOM Trichomonas test is not able to distinguish between *Trichomonas* species; however, results suggest that the test is suitable for the rapid detection of *Trichomonas* spp. infection in pigeons.

RESUMEN. *Nota de Investigación—Trichomonas* spp. en Palomas: Detección por la prueba rápida de OSOM para Trichomonas. Se investigó la eficacia de la prueba rápida OSOM para Trichomonas (desarrollada para el diagnóstico rápido de *Trichomonas vaginalis* en humanos) en la detección de *Trichomonas* spp. en palomas (*Columba livia*). Se recolectaron dos hisopos de la cavidad oral de 50 palomas de granja. El cultivo en el medio de Diamond para Trichomonas se utilizó como un método de referencia. De acuerdo con una determinación morfológica, *Trichomonas gallinae* era el único protozoario que se encontró, sin embargo, no se realizó un análisis molecular posterior. La prueba OSOM para Trichomonas fue positiva con 39 hisopos orales. En comparación con el método de cultivo, se identificaron tres muestras como falsas negativas y una como falsa positiva. Se determinó que la especificidad y la sensibilidad del ensayo eran de 93% y 90%, respectivamente. Mediante la prueba Kappa de Cohen, se encontró una fuerte concordancia entre los dos métodos de prueba ($\kappa = 0.7506$, 95% CI = 0.5162–0.9850). La prueba de Trichomonas OSOM no fue capaz de distinguir entre especies de *Trichomonas*, sin embargo, los resultados sugieren que la prueba es adecuada para la detección rápida de la infección por *Trichomonas* spp. en palomas.

Key words: *Trichomonas* spp., pigeon, OSOM Trichomonas Rapid Test

Abbreviations: OSOM Trich = OSOM Trichomonas Rapid Test

Trichomonas gallinae is a common protozoan parasite that is identified as a cause of trichomoniasis in columbid species worldwide (19). The disease clinically manifests with necrotic ingluvitis and can be asymptomatic to fatal course. It is a well-documented fact that *T. gallinae* is also found in predators that feed on pigeons (14,15,16). However, recent studies show that the parasite emerged as a novel infection of passerines, leading to, in extreme cases, epidemic mortality associated with significant declines of breeding populations (1,11,12,13).

Diagnosis of this infection can be made by direct microscopic examination of material scraped from the oral cavity or by the inoculation of such material into a suitable medium (10). Some authors (3,6) recommended the use of the InPouch™ TF (BioMed Diagnostics, Santa Clara, CA), a commercially available culture pack developed to diagnose *Tritrichomonas foetus*. However, all of these methods have some drawbacks; above all, they are difficult to implement in field conditions. Cultivation methods are time consuming and require some laboratory equipment.

Recently molecular techniques have been used to disclose the phylogeny of avian trichomonads (1,8). Some authors (9) suggested the existence of at least three clusters within the *T. gallinae* species complex, two groups being closely related to the human pathogens *Trichomonas vaginalis* and *Trichomonas tenax*.

The OSOM Trichomonas Rapid Test (OSOM Trich; Genzyme Diagnostics, Cambridge, MA) is an immunochromatographic capillary-flow enzyme immunoassay dipstick test that was developed for rapid diagnosis of human *T. vaginalis* (4). This test is easily performed according to the manufacturer's instructions and can be used in the field.

T. gallinae or *Trichomonas*-like organism infection spread among different species of birds. A method for the quick and easy testing of large numbers of birds for this infection in natural conditions can become a very efficient tool for detecting this disease in wild populations, as well as in domesticated birds. Thus, the aim of present study is to investigate the efficacy of the OSOM Trich for the detection of *Trichomonas* spp. in pigeons.

MATERIALS AND METHODS

Fifty pigeons (*Columba livia)* from two different farms in the Czech Republic were examined for *Trichomonas* spp. infection by two different methods. Two subsequent oral cavity swabs were taken from each individual at the same time. The first samples were placed into Diamond

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Table 1. Comparison of OSOM Trichomonas Rapid Test to cultivation in Diamond Trichomonas medium.

		Cultivation		
		+	−	Total
OSOM Trich	+	38	1	39
	−	3	8	11
	Total	41	9	50

Trichomonas medium (OXOID, Basingstoke, UK) and transported to the laboratory (a reference method), while the second ones were immediately examined on location by the OSOM Trich.

The Diamond medium was stored at 4–8 C and pre-heated to 39 C prior to use. The medium was then dispensed in 2 ml aliquots in capped plastic culture tubes and placed into a transferable thermo box. The oral cavities of pigeons were swabbed with dry cotton-tipped pre heated swabs. The cotton tip of the swab was cut off, placed into medium-filled culture tubes thermo box. The culture tubes were closed, labeled, and transported to the laboratory, where they were vertically incubated at 39 C for 5 days. Afterwards, thin culture smears were made from all culture tubes and air dried. The air-dried smears were fixed in methanol, stained with Giemsa and microscopically examined for the presence of flagellated protozoans with both lower (200×) and higher powers (400×, 1000×). Trichomonads were identified according to previously published description (2,18).

The OSOM Trich was performed according to the manufacturer's instructions. A positive result displays both a red internal control and a blue positive test line, while the negative result displays only a red internal control line. Invalid tests display an absent internal control line. The analysis required 10 to 15 min to carry out.

Statistical computations were performed with Statistica 10 software. The level of agreement between the two tested methods was measured by Cohen's Kappa (5). A κ value higher than 0.75 indicates a strong level of agreement (7). The test sensibility and specificity were also computed.

RESULTS AND DISCUSSION

In this study 50 pigeons (*Columba livia)* were examined for the presence of *Trichomonas* spp. infection. According to a morphological determination of cultivated oral swabs, *T. gallinae* was the only protozoan found; however, no further molecular analysis was conducted. By using the OSOM Trich, *Trichomonas* spp. was found in 39 oral swabs. In comparison with cultivation in Diamond Trichomonas medium used as a reference method (a golden standard), three samples were identified as false negative and one as false positive (heavy fungal overgrowth, but no trichomonands were found in contaminated culture). However, the OSOM Trich performed very well (Table 1). Using Cohen's Kappa, the concordance between two testing methods was found to be strong (κ = 0.7506, 95% CI: 0.5162–0.9850). Furthermore, the OSOM Trich had satisfactory sensitivity (93%, 95% CI: 85.9%–100%) as well as specificity (90%, 95% CI: 81.7%–98.3%).

Usually those who wish to test pigeons for trichomonad infection rely upon diagnostic methods, such as direct microscopic examination, or on the inoculation of scraped material into a suitable medium. However, in many cases, the lack of an experienced microscopist precludes accurate trichomonad detection. The material scraped from oral cavities of birds must be transported to a microbiology laboratory and examined by technicians, which could cause a significant time delay. The sensitivity of direct microscopic examination in detecting trichomonands declines substantially with even relatively short intervals between collection and examination (15). For the diagnosis of *T. gallinae* in wild birds, some authors (3,6) have recommended the InPouch TF commercially available culture system, which combines immediate viewing and sample culturing. Despite having favorable results and costing half the price of the OSOM Trich, the InPouch TF system requires 72 hr (3) of incubation to provide an accurate diagnosis; this disadvantage can make it quite difficult to quickly and effectively inspect large numbers of individuals in the field. Similar drawbacks can also be found in the use of very accurate molecular methods to diagnose trichomoniasis (17).

This study is the first to confirm the application of the OSOM Trich to identify *Trichomonas* spp. infection in pigeons based on the use of oral cavity swabs. The OSOM Trich is not able to distinguish between the *Trichomonas* species; however the sufficient sensitivity and specificity of the test as well as the swiftness and simplicity of its performance make the OSOM Trich an ideal tool for Trichomonas spp. testing by even inexperienced personnel both in the field and in poultry breeding facilities.

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3.4 Příspěvky ve sborníku

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3.5 Dosud nepublikované výsledky

1) Vylučování zinku močí u potkanů předávkovaných mléčnanem zinečnatým a infikovaných tasemnicí

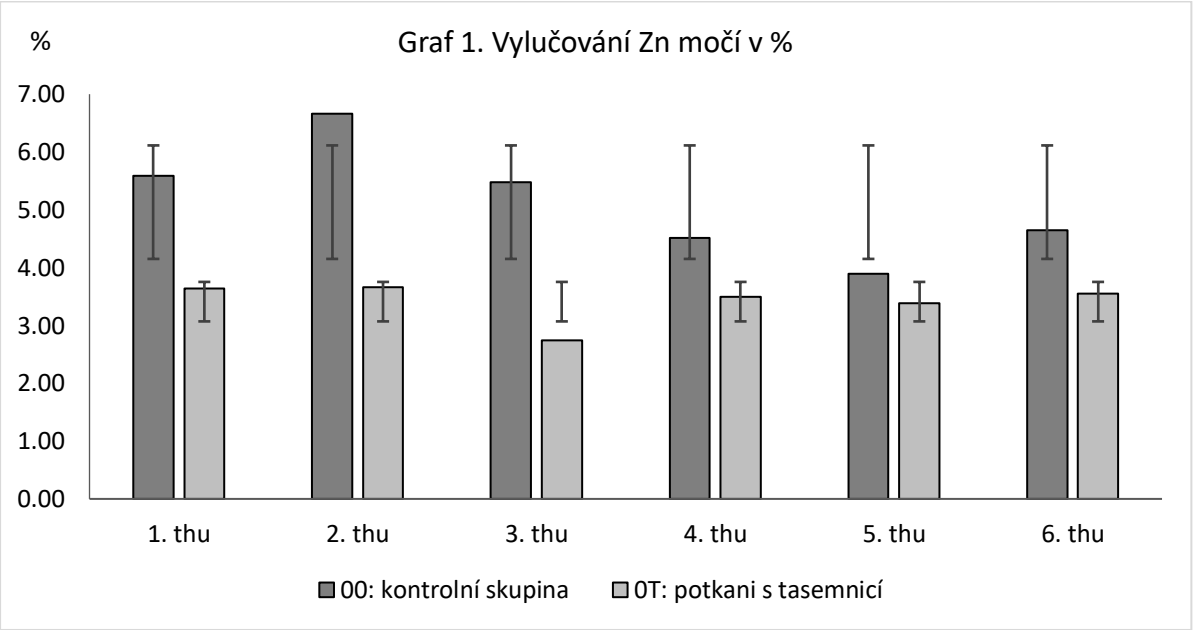
Tabulka 1. Obsah zinku v krmivu a rozdělení skupin

Označení skupin	Počet jedinců	Přítomnost tasemnice	Obsah zinku (mg/25g krmiva)	Množství zinku podaného za týden každému jedinci (mg)
00	6	-	1.75	10.5
0T	6	+	1.75	10.5
M0	6	-	20.5	123
MT	6	+	20.5	123

Tabulka 2. Množství přijímaného zinku potravou a vylučovaného zinku močí za dobu 6 týdnů trvajícího experimentu

Experimentální skupiny	Denní příjem zinku (mg/den)	Vylučování zinku močí (mg/den)	Vylučování zinku močí (%)
00	1.75 ± 0.13	0.082 ± 0.04	4.88
0T	1.70 ± 0.17	0.049 ± 0.03	3.43
M0	20.25 ± 0.14	0.629 ± 0.35	3.22
MT	19.23 ± 1.78	0.111 ± 0.09	0.61
Hodnoty jsou uvedeny jako medián ± směrodatná odchylka pro celou skupinu			

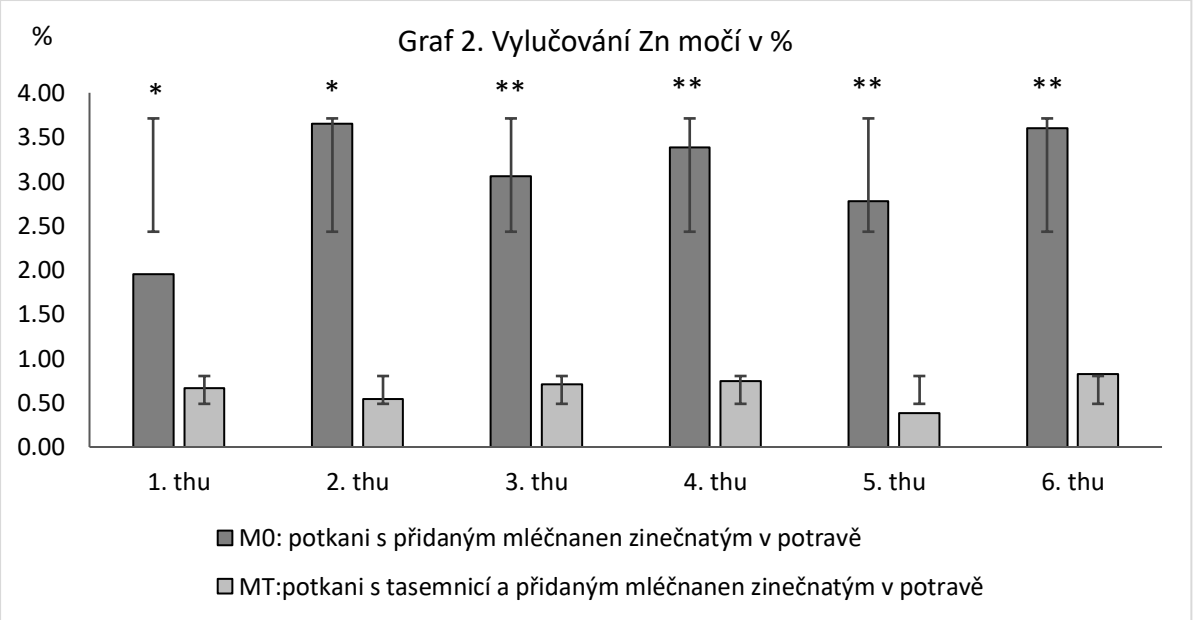
Graf 1. Srovnání množství vylučovaného Zn (%) v moči potkanů bez tasemnice (00) a potkanů infikovaných tasemnicí (0T)



* statisticky významný rozdíl mezi skupinami 00 a 0T, $p < 0.05$

** statisticky významný rozdíl mezi skupinami 00 a 0T, $p < 0.01$

Graf 2. Srovnání množství vylučovaného Zn (%) v moči potkanů bez tasemnice a přidaným mléčnanem zinečnatým v potravě (M0) a potkanů infikovaných tasemnicí a přidaným mléčnanem zinečnatým v potravě (MT)



* statisticky významný rozdíl mezi skupinami M0 a MT, $p < 0.05$

** statisticky významný rozdíl mezi skupinami M0 a MT, $p < 0.01$

4 Diskuze

Zinek je klíčový mikronutrient, přítomný ve všech orgánech, tkáních a tělesných tekutinách. Oproti zinku je kadmium výhradně toxickým kovovým prvkem. Vzhledem k velmi podobným fyzikálním a chemickým vlastnostem obou kovů dochází v organismu při souběžném příjmu k jejich záměně, což má významně negativní dopady na správné fungování mnoha pro život klíčových metabolických procesů. Hlavní cestou exkrece obou kovů je gastrointestinální trakt (Erdman et al. 2012). Velká část Zn je znovu resorbována ze střeva, čímž je udržována dostatečná hladina Zn v těle při různém příjmu. Krebs (2000) uvádí, že u lidí i zvířat jsou pro udržení zinkové homeostázy nejdůležitější dva mechanismy – endogenní střevní exkrece a množství zinku absorbovaného střevem. Zdroj kadmia v gastrointestinálním traktu je v podstatě shodný se zinkem. Menší část Zn (2–10 %) je vylučována močí. Z našich prozatím nepublikovaných výsledků studie zabývající se vylučováním zinku močí u potkanů krmených standardní směsí pro hlodavce ve srovnání s potkany krmenými rovněž touto směsí, ale obohacenou o mléčnan zinečnatý, kdy ještě byla zohledněna přítomnost tasemnice, můžeme říct, že hodnoty uvedené ve studii Krebs (2000) se shodují s našimi výsledky (tabulka 2, strana 150). Mezi kontrolními skupinami (00, 0T) nebyl zjištěn statisticky významný rozdíl v žádném z šesti sledovaných týdnů (graf 1, strana 150). Oproti tomu mezi skupinami pokusnými (M0, MT) byl zjištěn statisticky významný rozdíl ve vylučování zinku močí, kdy skupina potkanů infikovaná tasemnicí a krmená potravou s přidaným mléčnanem zinečnatým, vylučovala statisticky významně méně zinku ve všech šesti sledovaných týdnech než skupina potkanů krmená tou samou směsí, ale neinfikovaná tasemnicí krysí. V prvním a druhém týdnu dosáhl statisticky významný rozdíl mezi skupinami M0 a MT hodnoty ($p < 0,05$) a ve zbývajících čtyřech týdnech dokonce hodnoty ($p < 0,01$) (graf 2, strana 150).

Rostliny patřící mezi hyperakumulátory akumulují vysoká množství rizikových prvků, včetně zinku a kadmia. Mohou tak významným způsobem mobilizovat tyto kovy v potravním řetězci. Když zvířata spásají vegetaci, mohou také pozřít tyto rostliny. Tím se do těla živočichů může dostat značné množství rizikových prvků (Bothe & Slomka 2017; Zhao et al. 2006).

Tasemnice parazitující v trávicím traktu svého hostitele, jsou schopné akumulovat rizikové prvky ve svém těle. Tím mohou vstupovat do procesu absorpce rizikových prvků přijatých svým hostitelem. Autoři Sures et al. (2002) svými výzkumy dokázali, že díky velké akumulační schopnosti mohou tasemnice, vyskytující se u hlodavců, zejména *Hymenolepis diminuta* parazitující v potkanech *Rattus norvegicus*, ovlivňovat akumulaci kovů v těle hostitele. Kromě akumulace kovů v těle samotného parazita mohou tasemnice ovlivnit absorpci kovů ve střevech svého hostitele fyzickým působením na střevní stěnu. Autoři Martin & Holland (1984), zjistili souvislost mezi přítomností tasemnice *Hymenolepis diminuta* a stavem střevních klků. Dle jejich studie přítomnost tasemnice způsobuje rozsáhlou atrofii klků. Nejvíce extrémní změny v architektuře mukózy zjistili v blízkosti zralých článků tasemnice. V těchto místech byly střevní klky často redukovány na zploštělé struktury. Velikost tasemnice hrála důležitou roli, protože větší tasemnice poškodily klky více. Podle Hall et al. (2008) tasemnice narušují vstřebávání živin především v poslední třetině ilea, což může vést až k anemii. V oblasti ilea je navíc podle Hotz & Brown (2004) vstřebáváno přibližně 30 %, podle Johnson (1989) dokonce 60 % zinku přijímaného potravou.

Vylučování zinku a kadmia

Součástí předložené práce tedy bylo vyhodnotit vylučování zinku a kadmia výkaly a močí potkana (*Rattus norvegicus*) po přijetí těchto kovů ve formě potravy obsahující hyperakumulátor, *Arabidopsis halleri,* kdy část potkanů byla infikována tasemnicí (*Hymenolepis dimunuta*) (tito potkani označeni jako PT) a druhá část potkanů tasemnici neměla (tito potkani označeni jako P0). Dále také vyhodnotit vylučování zinku výkaly a močí v případě přijetí vysoké dávky tohoto kovu podané formou suplementu, mléčnanu zinečnatého v krmivu. I zde část takto krmených potkanů byla infikována tajemnicí (potkani označeni jako MT) a druhá část potkanů krmených touto směsí tasemnici neměla (potkani označeni jako M0). Vůči těmto pokusným skupinám (P0, PT, M0, MT) byla hodnocena kontrolní skupina potkanů krmená potravou bez přidaného zinku a kadmia, kdy část těchto potkanů byla infikována tasemnicí (potkani označeni jako 0T) a druhá část potkanů tasemnic neměla (potkani označeni jako 00). Podle Klaassen et al. (2009), je regulace vylučování zinku v gastrointestinálním traktu hlavním mechanismem pro udržení

zinkové homeostázy. Ve výkalech je neabsorbovaný zinek přijatý potravou, endogenní zinek vyloučený do střeva ze žlučníku a slinivky břišní, zinek vyloučený gastroduodenálními sekrety a Zn vyloučený z buněk mukózy (Erdman et al. 2012). Tasemnice tak přicházejí do styku s kovy přijímanými potravou a také kovy do střeva vyloučenými z těla.

Vylučování zinku a kadmia výkaly

Z výsledků studie Sloup et al. (2018) zabývající se vylučováním zinku a kadmia výkaly potkanů, vyplynulo méně vylučovaného zinku v prvních dvou týdnech především u skupin neinfikovaných tasemnicí a krmených potravou s příměsí *Arabidopsis halleri* (P0). Dá se předpokládat, že v těchto prvních dvou týdnech bylo více zinku absorbováno než v dalších týdnech z důvodu sycení kapacit v organismu. Studie King et al. (2000), potvrzuje různé množství zinku vylučovaného výkaly v závislosti na délce expozice. Zdá se, že množství vyloučeného zinku má vzestupnou tendenci v čase. Je možné, že v našem experimentu Sloup et al. (2018) v dalších týdnech potkani vylučovali zinek akumulovaný v prvních dvou týdnech, nebo schopnost organismu ukládat zinek byla naplněna v průběhu prvních dvou týdnů a v týdnech následujících již organismus aktivoval mechanismy, které omezovaly možnost zinek v trávicím traktu absorbovat.

V jiné studii autoři zjistili souvislost mezi přijatou dávkou zinku a zinkem vstřebaným, respektive vyloučeným výkaly. Wada et al. (1985) uvádí, že je vstřebáno asi 53 % zinku, když jeho dávka v potravě činí 16,5 mg/den a 49 %, když je v potravě 5,5 mg zinku denně. Naopak Jackson et al. (1984) uvádí, že s každým zvýšením obsahu zinku v potravě kleslo množství absorbovaného zinku. Z počátečních 58 % absorbovaného zinku z potravy došlo k poklesu na 47 % po 4 dnech. Když bylo množství zinku v potravě zdvojnásobeno, absorpce byla během prvních 4 dnů na hodnotě 45 % a v následující 4denní periodě klesla až na 32 %. Také další studie dokazuje velkou schopnost organismu regulovat příjem zinku. Z potravy chudé na tento kov absorboval organismus až 100 % zinku. Pokud bylo potkanům potravou podáváno 0,5 mg Zn/den, klesla absorpce na 55 % (King et al. 2000). V naší studii Sloup et al. (2018) potkani z kontrolních skupin (00, 0T), kdy přijímali maximálně 1,75 mg Zn denně, vylučovali 67 až 79 % zinku výkaly. Z toho můžeme předpokládat, že absorpce se pohybovala v rozmezí 21-33 %.

Potkani zařazení do pokusné skupiny M0 vylučovali výkaly průměrně 77 % zinku, přičemž nejvíc zinku vyloučili poslední týden – 94 %. U skupiny MT naopak bylo nejvíce vyloučeného zinku výkaly v prvním týdnu (74 %), což by mohlo souviset s přítomností *Hymenolepis diminuta*, respektive její velikostí, která v průběhu experimentu rostla. Zároveň skupina MT vyloučila v průměru za celou dobu jen 65 % Zn, což naznačuje významný vliv tasemnice na vylučování zinku výkaly u této pokusné skupiny. Potkani zařazení do těchto pokusných skupin (M0, MT) přijímali maximálně 20,5 mg Zn denně.

Potkani zařazení do skupin krmených stravou s příměsí *Arabidopsis halleri* (P0, PT) také přijímali maximálně 20,5 mg Zn denně a výkaly vylučovali 71-91 % zinku. V trávicím traktu tedy absorbovali 9-29 % zinku přijatého potravou. To potvrzuje, že organismus na vyšší obsah zinku v potravě reaguje jeho vyšším vylučováním ve výkalech a zároveň množství vylučovaného zinku může v čase růst zvýšením endogenní sekrece a snížením absorpce, pokud je organismus dlouhodobě vystaven vysokým dávkám Zn.

Autoři King et al. (2000) popisují také schopnost organismu udržet obsah zinku na konstantní hladině přibližně 30 mg Zn/kg živé váhy, zatímco příjem zinku potravou se liší třeba až 10krát. Pokud příjem zinku potravou klesl pod 10 mg či stoupl nad 100 mg v kilogramu potravy, homeostatické mechanismy již neudržely stabilní hladinu zinku v těle. V takovém případě došlo při nižším příjmu (<10 mg) zinku ke snížení jeho celkového množství v těle, zatímco při vyšším příjmu (>100 mg) došlo ke hromadění zinku v tkáních. V naší studii Sloup et al. (2021a) zabývající se vylučováním zinku výkaly, byl obsah zinku v krmivu zvolen následovně: pro skupiny kontrolní (00 a 0T) činil 70 mg zinku na kilogram potravy a pro skupiny pokusné (M0 a MT) 820 mg Zn/kg potravy. Obsah zinku v krmivu kontrolních skupin tedy odpovídal rozmezí, kdy podle King et al. (2000) je organismus schopen udržovat konstantní obsah zinku v těle, zatímco u skupin pokusných bylo toto rozmezí výrazně překročeno. Tasemnice neovlivnila množství vylučovaného zinku ve výkalech u kontrolní skupiny (0T) do té míry, aby byl zjištěn statisticky významný rozdíl (Sloup et al. 2021a, graf 1). Oproti tomu u pokusné skupiny (MT) byl zjištěn statisticky významný rozdíl ve všech týdnech, kromě prvního (Sloup et al. 2021a, graf 2).

Podle Davies & Nightingale (1975), je výkaly vylučováno přibližně 70-80 % požité dávky zinku. V naší studii se množství zinku vyloučeného výkaly za 24 h

pohybovalo v rozmezí 67-79 % u skupin 00 a 0T (Sloup et al. 2018, graf 1) a 71-91 % u skupin P0 a PT (Sloup et al. 2018, graf 2). To ukazuje na relativně vyrovnané vylučování zinku. Nicméně skupiny P0 a PT přijímaly 12krát více zinku než skupiny 00 a 0T. To může souviset s lepší absorpcí zinku vázaného v rostlině, což by však bylo v rozporu se studií House et al. (1982), kteří uvádějí, že kyselina fytová je považována za antinutriční látku, snižující absorpci některých látek včetně zinku u zvířat i lidí z potravy.

Obdobně v případě kadmia potvrzují autoři Cikrt & Tichý (1974) rozdíl v množství vylučovaného kadmia v případě různě velkých přijatých dávek v potravě. Zatímco z přijaté dávky 67 μg bylo vyloučeno za 24 hodin 0,83 %, z 90 μg 1,18 % a z 120 μg už 5,68 % kadmia. V naší studii mohli potkani přijmout maximálně 0,41 mg kadmia denně. Hodnota vyloučeného kadmia se pohybovala v rozmezí 71-82 % u skupiny P0 a mezi 58-73 % u skupiny PT (Sloup et al. 2018, graf 3). Rovněž je zde také patrný významný rozdíl v množství vyloučeného kadmia výkaly mezi potkany bez tasemnice a potkany s tasemnicí, kdy potkani infikovaní tasemnicí vylučovali výkaly méně kadmia než potkani bez tasemnice. Decker et al. (1957) podali potkanům 2 mg kadmia orálně, tedy přibližně 5krát víc, než jsme potkanům podávali denně v naší studii. Zjistili, že více než 90 % podané dávky bylo vyloučeno výkaly a pouze malé procento se vstřebalo. To potvrzuje vztah mezi velikostí přijaté dávky, absorpcí a množstvím vyloučeného kadmia výkaly. Podle Godt et al. (2006), je prostřednictvím gastrointestinálního traktu absorbováno přibližně 5 % požitého množství kadmia. To však závisí na přijaté dávce kadmia a nutričním složení potravy. V naší studii Sloup et al. (2018) činila absorpce maximálně 42 %, což je podstatně víc, než uvádí výše zmínění autoři.

Kadmium a zinek jsou prvky z chemického a fyzikálního hlediska velmi podobné (Das et al. 1997; Chaney 2010; Tang et al. 2014). To je důvod, proč se v těle vzájemně ovlivňují. Po absorpci v trávicím traktu se oba kovy váží na totožné bílkoviny – albumin v krevním řečišti, metalothioneiny (MT) a další bílkoviny v tkáních (Brzóska & Moniuszko-Jakoniuk 2001). Vysoký příjem Zn a Cd stimuluje syntézu na cystein bohatého MT ve sliznici tenkého střeva (Kägi 1991). Vazba na MT snižuje toxické účinky těchto dvou kovů, tedy především kadmia (Kelly et al. 1996). Podle autorů Funk et al. (1987) syntéza MT ve formě Cd_5Zn_2MT vede k přesunu této formy MT z krevní plazmy do jater a ledvin a následnému vylučování kovů z jater do střeva.

Vzhledem k tomu, že potkani ze skupin P0 a PT byly vystaveni oběma kovům zároveň, můžeme uvažovat nad možností, že do střeva byly oba kovy vylučovány v jiné formě, než u kontrolních skupin (00, 0T). Kovy v této formě mohly být tasemnicí lépe přijímány. To by vysvětlovalo větší rozdíly ve vylučování Zn výkaly mezi potkany infikovanými tasemnicí a potkany neinfikovanými tasemnicí u skupin P0 a PT oproti skupinám 00 a 0T (Sloup et al. 2018, graf 1 & 2). Tato úvaha je podpořena zjištěním, že různí gastrointestinální helminti akumulují různé kovy v odlišném množství. Například hlístice *Haemonchus placei* obsahuje vysoké množství mědi, motolice *Gastrothylax crumenifer* a hlístice *Ascaris lumbricoides* zase železa. Hlístice *Haemonchus contortus* vysoké množství kobaltu a například tasemnice *Diphillobothrium latum* selektivně absorbuje vitamin B12 ze stravy svého hostitele, čímž snižuje jeho množství dostupné pro organismus hostitele. V těle parazita se tedy koncentruje také značné množství kobaltu (Theodor Von Brand 1973). Svou roli také může hrát fakt, že zinek je v rostlinných hypearkumulátorech vázán především jako komplex Zn-organická kyselina (Isaure et al. 2015) a může tak být parazitem lépe přijímán než zinek přidaný do základního krmiva ve formě soli zinku.

Podle Theodor Von Brand (1973), obsahuje tělo tasemnice okolo 19 % anorganických látek. Z výsledků naší studie Sloup et al. (2018) vyplývá podstatně větší vliv tasemnice na vylučování kadmia než zinku výkaly u svého hostitele. To může být způsobeno vyšší afinitou k příjmu kadmia než zinku. Nicméně zinku exponované skupiny (P0, PT) dostávaly podstatně víc než kadmia. Stačil tak i relativně malý příjem kadmia tasemnicemi, aby došlo k výraznému rozdílu mezi skupinou infikovanou tasemnicí a skupinou bez tasemnice.

Akumulace zinku a kadmia v tkáních

Tkáně jsou důležitou součástí metabolismu zinku a kadmia a procesů udržujících zinkovou homeostázu. Funkce tkání je zásadní během příjmu, akumulace i vylučování obou těchto kovů (Lee et al. 1993; King et al. 2000).

Ve studii Brozova et al. (2015) byla koncentrace kadmia v tkáni tenkého střeva lišek (*Vulpes vulpes*) infikovaných tasemnicí *Echinococcus multilocularis* nižší než u lišek bez tasemnice. Stejně tak koncentrace zinku v tkáni tenkého střeva lišek bez tasemnice byla vyšší než u lišek s tasemnicí.

Jankovská et al. (2010) ovcím podávali orálně kadmium. Zjistili vyšší koncentraci kadmia v tasemnici oproti všem sledovaným orgánům ovcí kromě jater. Také zjistili, že ovce infikované *Moniezia expansa* měli koncentraci kadmia v tkáních vyšší než ovce bez tasemnice.

Ve studii Jankovská et al. (2016) jsme porovnávali koncentrace zinku a kadmia mezi tkáněmi u jednotlivých skupin potkanů. Koncentrace Cd byly významně vyšší u potkanů, kterým byla podávána *Arabidopsis halleri* v jejich krmné směsi (skupina P); tato skupina (P) měla hladiny Cd 329; 147; 87; 39; 10 a 3krát vyšší v ledvinách, játrech, tenkém střevo, varlatech, slezině a svalové tkáni oproti potkanům, kterým *A. halleri* nebyla v krmné dávce podávána (skupina C).

Hladina Zn v organismu a tím i vyloučeného Zn je přímo ovlivněna obsahem zinku ve stravě. Výkaly obsahují nevstřebaný zinek z potravy, endogenní zinek vyloučený do střeva ze slinivky břišní a žlučníku a zinek z buněk střevního epitelu (Krebs 2000). Játra, ledviny a kosti potkanů ve studii Jankovská et al. (2016) zařazených do skupiny TC, obsahovaly významně nižší koncentrace Zn ($p < 0,01$) než stejné tkáně potkanů zařazených do skupiny C; Játra, ledviny, svaly, kosti, varlata a slezina potkanů infikovaných tasemnicí a krmených *A. halleri* (TP) měly významně ($p < 0,01$) nižší koncentrace Cd, než tomu bylo u potkanů bez tasemnice (P). Ve skupině TP tasemnice akumulovaly koncentrace Zn, které byly 1,8; 2,3; 1,9; 1,4; 1,7; 1,2 a 5,5krát vyšší než koncentrace Zn akumulované v játrech, slezině, ledvinách, kostech, tenkém střevě, varlatech a svalové tkáni potkanů. Koncentrace Zn tak byly nižší ve všech tkáních hostitele ve srovnání s koncentrací v tasemnici.

Scheef et al. (2000) popsali schopnost parazita, vrtejše *Moniliformis moniliformis*, hromadit kadmium ve svém těle ve vyšších koncentracích oproti tkáním svého hostitele, potkana. Studie trvala 3 týdny a potkani byli vystaveni roztoku $CdCl_2$. Autoři zjistili, že parazit nahromadil ve svém těle podstatně více kadmia než tkáně potkanů. Podobné výsledky publikoval Sures et al. (2000), tentokrát v případě olova, dalšího rizikového prvku. Autoři zjistili, že parazit *M. moniliformis*, parazitující v potkanech, akumuloval vysoké koncentrace Pb z těla svého hostitele. Samice *M. moniliformis* obsahovaly 25; 39; 2 a 9krát více Pb než játra, tenké střevo, kůra nadledvin, respektive dřeň ledvin potkanů. Poměr koncentrace Pb v parazitovi a tkáních hostitele u samců *M. moniliformis* byl jiný (7; 11; 0,5 a 3). Tasemnice jsou však na rozdíl od vrtejšů hermafroditi. Ve naší studii byla skupina potkanů zařazená

do skupiny TP vystavena vysokým dávkám kadmia prostřednictvím potravy a koncentrace Cd v tasemnici u této skupiny byly 2,2; 32,0; 127,6; 2,7; 59,6 a 1551,6krát vyšší než v játrech, slezině, kosti, tenkém střevě, varlatech a svalové tkáni hostitele (Jankovska et al. 2016, tabulka 4). Ledviny jsou hlavním orgánem akumulujícím Cd u savců. To bylo naší studií potvrzeno, neboť koncentrace Cd v ledvinách dosáhla v případě skupiny TP úrovně 7,94 mg kg^{-1}, což bylo 1,6krát vyšší než koncentrace v tasemnici (Jankovska et al. 2016, tabulka 4).

Také Sures et al. (2002) studovali vliv tasemnice (*H. diminuta*) na množství olova v hostiteli, potkanovi. Potkanům bylo podáváno olovo ve formě $Pb(CH_3COO)_2$. Po vypočtení biokoncentračního faktoru autoři zjistili koncentrace olova v tasemnicích 17krát vyšší než koncentrace v ledvinách potkanů.

Ve naší studii jsme zjistili koncentrace Zn u tasemnic 1,9krát vyšší než v ledvinách hostitele (Jankovska et al. 2016, tabulka 3). Naopak koncentrace Cd v ledvinách hostitele ze stejné skupiny (TP) byly o 2,85 mg kg^{-1} vyšší než ty v tasemnicích (Jankovska et al. 2016, tabulka 4). I přesto publikované výsledky ukázaly, že tasemnice mají významný vliv na akumulaci zinku a kadmia v hostiteli. Tasemnice akumulovaly více zinku a kadmia než většina hostitelských tkání. Například tasemnice akumulovala 5,5krát více Zn a 1552krát více Cd než svalová tkáň hostitele. Při hodnocení akumulace Cd v tkáni tasemnice bylo zjištěno, že tasemnice u potkanů ze skupiny TP akumulovaly 848krát více Cd ve své tkáni než tasemnice ze skupiny TC.

Pro správné pochopení vztahu mezi vlivem tasemnice na příjem a vylučování zinku a kadmia výkaly a močí a vlivem tasemnice na akumulaci těchto kovů v tkáních hostitele, je nezbytné poznat také vliv tasemnice na biochemické parametry hostitele. To je předmětem naší studie Sloup et al. (2021), která rovněž rozšiřuje dosavadní znalosti o vlivu Zn/Cd hyperakumulátoru, rostliny *A. halleri*, na organismus potkana a také zkoumá účinky dalšího faktoru ovlivňujícího fyziologii těchto pokusných zvířat. Tím faktorem je předávkování potkanů infikovaných i neinfikovaných *H. diminuta* laktátem zinečnatým v potravě.

H. diminuta je považována za parazita vhodného k léčebné metodě nazvané helmintoterapie (Kosik - Bogacka et al. 2010, 2016; Wegener Parfrey et al. 2017). Nejprve jsme porovnali změny v různých biochemických parametrech u skupin 00 a 0T. Pouze celková bílkovina, močovina a hladina fosforu byly významně vyšší

v séru potkanů infikovaných tasemnicí oproti (0T) potkanům bez tasemnice (00). Ostatní parametry (albumin, glukóza, triacylglyceroly, neesterifikované mastné kyseliny, cholesterol, alkalická fosfatáza, aspartát aminotransferáza, kyselina močová, hořčík, vápník a zinek) nebyly významně ovlivněny přítomností tasemnice. To podporuje tvrzení Wegenera et al. (2017), že *H. diminuta* je benigní střevní probiotický helmint, který by mohl být využíván pro potřeby helmintoterapie. *Hymenolepis diminuta* se tak zdá být zajímavým modelem pro helmintoterapie, protože dlouhodobě a stabilně kolonizuje gastrointestinální trakt potkanů a moduluje imunitní systém, aniž by způsobila bakteriální dysbiózu. Výrazně vyšší hladina celkového proteinu u potkanů s tasemnicí (0T) může být způsobena stavem, kdy se vlivem přítomnosti tasemnice zvýší množství imunoglobulinů. To může být využito k léčbě autoimunitních onemocnění (Wangchuk et al. 2019).

Tasemnice, stejně jako ostatní živočichové, potřebují zinek pro správnou funkci životně důležitých metabolických procesů, a proto jej absorbují z potravy přijaté hostitelem. Pro tasemnici je zinek nezbytný z hlediska vývoje, růstu a metabolismu bílkovin. Horakova et al. (2017) uvádí nejvyšší koncentrace zinku u tasemnice v nezralých proglotidech za scolexem. Je možné, že důvodem pro vyšší koncentraci zinku v těchto mladých článcích je účast zinku na procesu transkripce DNA, translace RNA a také procesu buněčného dělení. Autoři Goodchild & Wells (1957) analyzovali vzorky těla tasemnic *Hymenolepis diminuta* a zjistili významnou přítomnost histidinu i cysteinu. Také Pappas a Durka (1994) zjistili významné zastoupení histidinu a cysteinu v těle tasemnic. Z jejich výsledků vyplývá, že vaječný obal tasemnice *Hymenolepis diminuta* je asi z 32 % složen z bílkovin, přičemž histidin tvořil 22 % objemu aminokyselin. Vzhledem k významné přítomnosti histidinu a cysteinu v těle tasemnic, a provázanosti obou aminokyselin se zinkem, se dá předpokládat potřeba tasemnice zinek absorbovat a využívat podobně, jako je využíván u ostatních živočichů. Navíc při relativně značné produkci vajíček i neustálé obnově článků se můžeme domnívat, že tasemnice zinku potřebuje značné množství.

5 Závěr

Zinek patří, svým zastoupením v široké škále metabolických procesů nezbytných pro život, mezi jednoznačně nejvýznamnější esenciální biogenní kovy v živočišném organismu. Naopak z fyzikálně-chemického pohledu zinku velmi podobné kadmium je kovem výhradně toxickým. Právě velká podobnost obou kovů je důvodem vysoké toxicity kadmia. To je totiž v případě jeho příjmu organismem často za zinek zaměňováno, čímž dochází k zastavení životně důležitých metabolických procesů. Záměna kadmia za zinek vede k nefunkčnosti mnohých metaloproteinů či neschopnosti organismu produkovat inzulin.

Strava lidí i potrava hospodářských zvířat je mnohdy obohacována o suplementy. Tyto dobře vstřebatelné chemické sloučeniny jsou do potravy přidávány za účelem zvýšení množství důležitých nutrientů. Mezi takové látky patří také mléčnan zinečnatý. Hospodářská zvířata, ale i lidé, mohou značné množství rizikových prvků přijmout pozřením rostlin, které jsou schopné těchto prvků akumulovat značné množství. Mezi takové rostliny patří huseníček Hallerův z čeledi brukvovitých, přičemž do této čeledi řadíme řadu zemědělsky významných plodin.

Vzhledem k tomu, že zinek i kadmium jsou přijímány především absorpcí v gastrointestinálním traktu, je důležité zohlednit všechny faktory, které tento proces mohou ovlivnit. Velmi významným faktorem, který absorpci v gastrointestinálním traktu ovlivňuje, jsou parazité, konkrétně helminti. Tito paraziti v řadě případů dosahují ve vztahu ke svému hostiteli významné jak velikosti, tak hmotnosti. Kromě velikosti svého těla a s ní související potřebě nutrientů ovlivňují absorpci také fyzickým působením na střevní stěnu, tedy klky, čímž snižují aktivní povrch střeva a tím plochu pro vstřebávání živin do krve. Mezi helminty parazitující v gastrointestinálním traktu a mnohdy dosahující skutečně značné délky, patří tasemnice.

Byla publikována řada studií, které se zabývaly vlivem parazitů na akumulaci rizikových prvků v hostiteli. Předložená práce je však specifická svým komplexním pojetím daného problému. Vyhodnoceno bylo množství zinku a kadmia vyloučeného výkaly a močí hostiteli *(Rattus norvegicus*) bez tasemnice a hostiteli infikovanými tasemnicí krysí (*Hymenolepis diminuta*) po podání krmiva obohaceného o vysoké množství zinku ve dvou formách – krmiva obohaceného o suplement mléčnan

zinečnatý a hyperakumulující rostlinu, huseníček Hallerův (*Arabidopsis halleri*). Dále bylo vyhodnoceno množství obou kovů v sedmi vybraných tkáních potkanů i samotné tasemnici. Rovněž byl vyhodnocen vliv vysoké dávky zinku v krmivu na biochemické parametry potkanů.

Z výsledků je patrná značná schopnost tasemnice ovlivnit množství jak zinku, tak kadmia ve výkalech i moči potkana a rovněž schopnost tasemnice ovlivnit množství obou kovů v orgánech. Také byla zjištěna relativně vysoká koncentrace zinku i kadmia v těle tasemnice ve srovnání s tkáněmi hostitele. Zdá se tedy, že tasemnice hromadí kovy ze střevního obsahu. Zda tasemnice vstupuje do tohoto procesu před absorpcí kovu ve střevě hostitele, nebo zda akumuluje až kovy vyloučené z těla hostitele zpět do střeva, není jasné. Pravděpodobně se jedná o kombinaci obojího. Jaký je hlavní způsob, kterým do tohoto procesu tasemnice vstupuje, je téma pro další studie.

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