CHEMICAL ELEMENTS IN TISSUES AND HISTOLOGICAL CHANGES IN TISSUES OF HARES (*L. EUROPAEUS* PALL.)

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Contamination of selected parenchyma organs, skeletal muscles of hares, chemical elements and histological defects of tissues in conditions of anthropogenically exposed locality Týnec nad Sázavou were studied. This site is located in northern edge of the Benešov district, it is broken with general north-western inclination. In view of geology it lies in north-western part of Central Bohemian pluton. It is built by amphibolic granodiorite to quartz diorite. In addition, there are gabbros. Local sources of pollution in agglomeration Týnec are represented by the Metallurgical Plant (ferrous and chromatic metallurgy) railway line and road No. 106. In a studied set of free-living hares higher concentration of arsenic was observed in parenchyma organs and in muscles in republic's comparison, the other concentrations of elements were lower in this comparison. In cadmium the highest value was recorded in liver and kidneys, less in muscles. Higher percentages of pathomorphological findings compared with long-time average were recorded in brain. They were classified as tiny nonspecific changes. Moreover, defects in liver of various range, character and change in spleen and kidneys of different intensity were recorded. Affection of gastrointestinal tract of the studied set was lower, evidently due to the fact that evaluation came from short period of winter (November, December, January). In hares no changes were found neither in testicles, nor in epididymides. In female hares urogenital tract was affected comparatively more compared with other sites.

hares; tissues; chemical elements; contamination; histological defects

INTRODUCTION

Free-living vertebrae, particularly the game, are a suitable bioindicatory factors of selected types of anthropogenic load of the landscape (Páv, 1985; Nováková, 1987; Bukovjan et al., 1988; Bukovjan, Páv, 1989; Černý, 1995). The goal-directed health condition of hare populations was in the centre of attention in the Czech Republic by Páv (1985) and Bukovjan et al. (1993), in Slovakia by Ciberej (1992) and Slamečka (1993). Histological examination of hare game was evaluated by Karpenko and Bukovjan (1992), including the occurrence and casuistic description of tumorous diseases, followed by Šebová (1990), Hoffman, Morl (1985) and Flux (1965) who dealt with the problems of reproduction.

The problems of occurrence of chemical elements, including the group of the so-called hazardous heavy metals in hares, were sufficiently processed. One of the first contributions can be considered the study written by Páv et al. (1982) which sums up the concentrations of elements in the game obtained by the AAS method. The identical problems were later dealt also by Tota (1987), Páv, Márová (1988), Maňkovská, Chudík (1987), Kačúr (1990), Kučera (1991) and Bukovjan et al. (1995). In terms of the goal-directed monitoring of the hygienic control of contamination of game by foreign substances within SVS CR (Collective of authors, 1994, 1995). An overview of the situation in the region of load of organs and muscles of the game and of its health condition are presented in the Yearbook of the Ministry of Economy of CR (Bukovjan, Šebesta, 1989; Bukovjan et al., 1995; Černá, Hrabětová, 1995, 1996).

The study was aimed at finding the level of contamination of selected parenchyma organs, skeletal muscles, selected set of 15 hares by chemical elements and histological defects of tissues in the years 1988 to 1995 in conditions of anthropogenically exposed locality Týnec nad Sázavou (Benešov near Praha district) with specific sources of contamination (METAZ – metallurgical plant, the JAWA Company).

MATERIAL AND METHOD

Toxicological examination

Hares were caught in immediate surroundings of Týnec nad Sázavou in the period of full hunting, i.e. in November and December, or January where this game was caught, and redundant males were offered for the research purposes. Their age was determined according to routine field methods (development and condition of external sex organs, presence of Stroh's outgrow, ossification of skeletal skeleton). In the case of controversial conclusions lower jaws were histologically processed and age of hares was determined by periosteal lines. 15 adult animals in total were examined from the given locality, of which the set of game presenting the period of 1988 to 1995 was determined.

Excisions of tissues for toxicological, parasitological and histological examination were collected during pathoanatomic examination which was done soon after caught, usually within 30 minutes (Bukovjan et al., 1993). Samples of the liver, both kidneys and skeletal muscles of the hind leg (the group of gluteal muscles) were analyzed for the presence of chemical elements after the previous mechanical homogenization and subsequent mineralization by the so-called wet way by AAS method on the device Varian SpectrAA 30P.

The mercury was determined by single-purpose detection apparatus AMA 254 without respective mineralization. The liver, kidneys and muscles determined for toxicological examination were to the time of the proper processing separately frozen at –21 °C.

Histological examination

Samples of hare organs (brain, lungs, heart, liver, spleen, kidneys, stomach, intestinal tract and reproduction organs) were fixed in 10% phormol for at least 48 hours and subsequently examined by routine histological procedures within paraffin method. Hematoxyl-eozine was chosen as a basic coloration, of special and selective colouring – Stein's method, Perls' ferrocyanide reaction, colouring after Gömöry, trichromium colouring etc.

To evaluate the results obtained standard mathematico-statistical methods were used. Indicators of hygienic safety of game and consumable organs of the game (Tab. IV) were evaluated by Hygienic regulations of the Ministry of Health of CR – Guideline No. 69 (1986).

RESULTS

Toxicological examination

Among the most contaminated tissue against the skeletal muscles were liver and kidneys which belong among the organs of parenchyma character. Higher average arsenic concentration (0.12 $mg.kg^{-1}$) was found in liver compared with kidneys (0.091 $mg.kg^{-1}$) and muscles (0.023 $mg.kg^{-1}$). The ratio between concentration of an element in the liver and muscles was 5.12 : 1,

that in kidneys and muscles was lower 3.95 : 1. Statistical evaluation is summed up in Tabs. I to III. Hygienic and health aspects of the game, kidneys and liver are summarized in Tab. IV.

Similar lower concentrations were found in the case of cadmium in muscles (0.155 $mg.kg^{-1}$) compared with liver and kidneys (Tabs. I and II). The ratio between loading of hepatic tissue and muscles was 6.90 : 1. Greater ratio was then between deposition of cadmium in kidneys and skeletal muscles (12.60 : 1).

In other hazardous chemical element, which is mercury in our ecosystems, average concentration in liver 0.132 $mg.kg^{-1}$, in kidneys 0.143 $mg.kg^{-1}$ and in skeletal muscles 0.020 $mg.kg^{-1}$ was found. The ratio between load of muscles and hepatic tissue was in this case 1 : 6.6, of muscles and kidneys it was roughly double (1 : 13.9).

In terms of lead, which can be considered as the best worked out hazardous element in toxicological sphere, the highest average concentrations were recorded in kidneys and liver of hares (Tabs. I and II). Significantly lower values of this element were detected in muscles (0.115 $mg.kg^{-1}$). The ratio between concentration in muscles and liver was comparable with the ratio between muscles and kidneys (1 : 3.47 and 1 : 3.59).

Of the group of biogenic elements, concentrations of copper and zinc in tissues of hares were investigated. Results of both elements are summarized in Tabs. I to III. In the studied locality in the set of examined animals copper and zinc concentrations exceeding still valid hygienic limit (Tab. IV) were not recorded. The ratio between concentrations in muscles and liver in the case of copper was 1 : 2.09, in muscles and kidneys 1:2.05. Similar in zinc concentrations were comparable between muscles and liver (1 : 1.60) and muscles and kidneys (1 : 1.56).

Histological examination

Within these detailed examinations, reversible and irreversible changes of various extent and character were recorded in hares. Results of the studied locality are confronted (Tab. V) with conclusions of long-time monitoring of health condition of hares in CR (Páv, 1985; Bukovjan et al., 1993; Černá, Hrabětová, 1995, 1996).

In the brain small nonspecific changes prevailed which in hemispheres in places circular-cellular perivascular infiltrations and mild cellular oedema were found. A rare finding was an inner fascicular oligo-dendrogliose and tygrolysis of the pons. The brain was totally affected in 20% of examined animals.

I. Concentration of chemical elements in liver of hares ($mg.kg^{-1}$)

		As	Cd	Cu	Hg	Pb	Zn
Liver	min.	0.0170	0.2800	2.9800	0.0090	0.0500	27.9300
	max.	0.5800	2.3800	8.0500	0.4060	0.6900	39.0400
	$\bar{x}$	0.1200	1.0700	4.6830	0.1320	0.3990	33.5990
	median	0.0425	0.9000	4.2100	0.1070	0.4000	33.8500
	$\pm s$	0.1544	0.5701	1.3029	0.1079	0.1851	3.3494
	Vk	363.27	63.347	30.948	100.85	46.264	9.8949
	10% quant	0.0202	0.5880	3.6960	0.0170	0.1340	29.4160
	90% quant	0.2342	1.8920	6.5460	0.2330	0.6360	37.4740

II. Concentration of chemical elements in kidneys of hares ($mg.kg^{-1}$)

		As	Cd	Cu	Hg	Pb	Zn
Kidneys	min.	0.0090	0.3800	3.070	0.0390	0.0500	27.7900
	max.	0.2200	4.6100	5.810	0.9930	0.7300	38.1200
	$\bar{x}$	0.0910	1.9540	4.589	0.2780	0.4130	32.8280
	median	0.0730	1.6900	4.610	0.2280	0.3900	31.8300
	$\pm s$	0.0720	1.2180	0.726	0.2210	0.1730	3.4470
	Vk	98.683	72.048	15.74	96.952	44.446	10.5140
	10% quant	0.0160	0.4940	3.726	0.0510	0.0244	29.2500
	90% quant	0.1990	3.3760	5.498	0.3940	0.6440	37.1200

III. Concentration of chemical elements in muscles of hares ($mg.kg^{-1}$)

		As	Cd	Cu	Hg	Pb	Zn
Muscle	min.	0.0050	0.0400	1.950	0.0050	0.0500	18.6300
	max.	0.0840	0.3100	2.780	0.0590	0.2000	23.1900
	$\bar{x}$	0.0230	0.1550	2.237	0.0200	0.1150	20.9860
	median	0.0110	0.1400	2.110	0.0110	0.1000	20.7900
	$\pm s$	0.0240	0.0840	0.240	0.0180	0.0460	1.39100
	Vk	220.151	60.325	11.38	161.79	46.278	6.68900
	10% quant	0.0050	0.0560	1.998	0.0050	0.0530	19.4520
	90% quant	0.0480	0.2780	2.516	0.0500	0.1780	22.9980

IV. Highest permitted concentrations of chemical elements in muscles and consumable organs of game (Regulations of the Ministry of Agriculture of CR No. 69/1986) and comparison of results proper (mg.kg^{-1} and %)

Element	As	Cd	Cu	Hg	Pb	Zn
Game	0.20	0.05	5.00	0.05	1.00	60.00
Above limit	20.0 %	73.3 %	0	6.7 %	0	0
Liver	1.00	0.50	60.00	0.10	1.00	80.00
Above limit	0	86.6 %	0	60.0 %	0	0
Kidneys	1.00	1.00	60.00	0.10	1.00	80.00
Above limit	0	73.3 %	0	80.0 %	0	0

V. Incidence of pathohistological lesions in studied organs of hare game (results given in %)

	Brain	Lungs	Heart	Liver	Spleen	Kidneys	Gastrointenstinal tract	Ovary
1981–1985	N	31.0	33.0	49.7	N	24.6	11.4–16.7	N
1986–1989	N	45.4	N	61.4	32.9	42.7	10.6–14.7	17.3
1989–1993	13.9	30.0	4.81	60.6	18.5	37.1	14.7–26.5	22.8
1995	12.2	34.1	7.32	61.0	29.3	37.1	31.70	20.0
1996	10.4	35.4	6.25	58.3	20.8	41.7	33.3	19.2
Týnec	20.0	26.6	0	73.3	26.6	40.0	13.3	25.0

N – not studied

In lungs changes which can be characterized as a direct consequence of catchinging (active congestion, oedema, emphysema) prevailed. Only in one case more marked cataral bronchopneumony of ill-defined reason was found. Verminous pneumony caused by pneumohelminths of the genus *Protostrongylus* was rarely diagnosed.

The most affected organ was the liver (73.3%). Here prevails a whole scale of slight nonspecific changes (significant lymphocytary infiltration, binucleate hepatocytes, hemosiderosis of Kupfer's cells, anisonucleosis) or starting degenerative changes. Small droplet form of steatosis, evidently as a result of noxious materials, followed by great droplet form of steatosis which can be classified as morphological and functional disorder of the liver of nutritional original. More significant proliferative changes of bile ducts and active congestion occurred.

Typical defects of the type of small wedge-shaped scars, usually accompanied by lymphocytary infiltrations of various extent. Hemosiderosis of kidneys was rarely detected, more frequent was the detection of degenerative changes of proximal tubules of various extent.

Except of mild bleedings hemosidorosis of various intensity was often found in the spleen. This organ was totally affected in 26% of examined samples of hares of the studied locality.

Catarrh enteritis was most frequently found in gastrointestinal tract. Mild bleedings were detected in stomach and erosion of the mucous membrane, probably due to the occurrence of parasites of the genus *Graphidium*. Intestinal parasites were found in polyvalent invasions, practically in 66.6% of animals of an examined set. Comparatively high was also the occurrence of intestinal coccidia of the genus *Eimeria* (86.6%). Coccidia usually occurred in mild and medium intensity of invasion.

Of reproduction organs no significant changes were detected in testicles of hare males. On the contrary, relatively abundant and diversiform were findings on ovaries of hare females (25%). Parovarial cysts were very frequent, usually localized in the hilum of ovary or follicular cyst. Inflammatory changes or imperforation of Fallopian tubes were rarely detected.

DISCUSSION

Hares are in our conditions considered as a suitable indication species within the conducted ecological monitoring of load of the landscape (Nováková, 1987; Páv, Márová, 1988; Bukovjan et al., 1993). Chemical elements, including hazardous heavy metals, are passing in a different amount through the placental barrier of female hares into developing foetuses and may participate uncontrollably in the genesis of tumorous diseases, decrease of immunity or hereditary defects (Karpenko, Bukovjan, 1992; Bukovjan et al., 1993). They are similarly eliminated by colostrum and later in the milk of hare females, and hence farther act in future development of young (Kučera, 1991).

In the studied set of hares from the locality Týnec nad Sázavou was found higher concentration of arsenic in parenchyma organs and muscles, compared with the nation-wide average. Others concentrations of chemical elements were lower against compared results. Maximum compared concentrations were in some elements significantly different (Černá, Hrabětová, 1996). The highest value of cadmium was recorded in liver 2.90 mg.kg^{-1}, in kidneys 8.49 mg.kg^{-1}, in muscles 0.63 mg.kg^{-1}, in mercury then 4.22, 8.90 and 0.96 mg.kg^{-1}. Very high concentrations of lead in tissues (liver 8.64 mg.kg^{-1}, in kidneys 3.63 mg.kg^{-1}, in muscles 3.11 mg.kg^{-1}) are probably

caused by secondary contamination due to the catching. In the studied set of hares from the region Týnec and Sázavou this situation can be fully eliminated. Concentration of this element can be therefore considered as natural, analogously to the concentration of biogenic elements. In samples secondary-contaminated by lead the values are usually as much as higher by two orders compared with our findings.

Loads of tissues of the game are corresponding to hygienic and health aspects of the game and consumable organs of the game. Comparatively high percentage of above-limit values against current results is presented in special literature (Páv, 1985; Tota, 1987; Páv, Márová, 1988; Bukovjan, Páv, 1989; Kačúr, 1990; Bukovjan et al., 1988, 1993; Collective of authors, 1995).

Comparatively higher percentage of findings against nation-wide average was recorded in the brain of hares originating from this locality. With respect to the fact that these findings were classified as slight nonspecific changes, predominantly of the cerebral cortex and less of the cerebral pons, the possibility of negative impact of some chemical elements in longer exposures and under-limit concentrations (As, Pb, Hg) can be concluded. Similar findings were detected earlier, e.g. in bioindicators from industrial Pardubice-Hradec Králové agglomeration. It has to be added that similar defects were previously described in hares in occurrence of the so-called subclinical form of aflatoxicosis B1 (Bukovjan et al., 1993).

Defects in the liver were of different extent and character. Against the results of the experimental localities (Černá, Hrabětová, 1996) this detoxication organ in hares from Týnec locality was more affected (73.3%). Pathomorphological lesions which prevailed can be classified as changes after ingoing regeneration processes of hepatic tissue due to action of noxious materials (Cd, Pb, mycotoxins) and as changes evidently degenerative (Štěrba, 1984; Kameník et al., 1992; Karpenko, Bukovjan, 1992; Bukovjan et al., 1993).

Some changes (different intensity of hemosidosis) in the spleen and kidneys indicate changes in deposition of blood stain hemosiderin in tissues at various forms of subclinical form of methemoglobinemia of hares which is still spread to a great extent in our conditions (Nováková, 1987; Páv, 1985).

Gastrointestinal tract was affected in the studied group less when compared with other results. This can be explained probably by the fact that evaluated animals originated from short period winter, while the other sets include the game of all the year round depistage catchings of game, including spring and late summer period when these animals have also very frequent digestive failures. To this corresponds also extensity and intensity of invasions of

gastrointestinal helminths and intestinal coccidia. Their occurrence and spectrum of species do not differ significantly from results of other authors.

No marked changes in testicles and epididymides were recorded in male hares. On the contrary, in female hares the urogenital tract was affected more compared with other localities. The occurrence of pathomorphological changes of analogous character ranged from 17.3 to 22.8% in the years 1986–1996 in the Czech Republic. Parallel results are given also from Slovakia (Šebová, 1990; Ciberej, 1992; Slamečka, 1993).

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Chemické prvky v tkáních a histologické změny tkání zaječí zvěře (*L. europaeus* Pall.).

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Byla posuzována zátěž vybraného souboru 15 kusů zaječí zvěře z období let 1988–1995 chemickými prvky. Byla vyhodnocena úroveň kontaminace chemickými prvky parenchymových orgánů a kosterní svaloviny zaječí zvěře a histologické defekty tkání v antropogenně exponovaném území se specifickými zdroji znečišťování. Bylo provedeno toxikologické a histologické vyšetření tkání.

Mezi nejvíce kontaminované tkáně oproti kosterní svalovině patřily játra a ledviny. V játrech byla zjištěna vyšší průměrná koncentrace arzenu (0,12 $mg.kg^{-1}$) ve srovnání s ledvinami (0,091 $mg.kg^{-1}$) a svalovinou (0,023 $mg.kg^{-1}$). Poměr mezi koncentrací prvku činil v játrech a svalovině 5,12 : 1, v ledvinách a svalovině byl nižší (3,95 : 1). Statistické vyhodnocení je shrnuto v tab. I až III.

Nižší koncentrace byly zjištěny v případě kadmia ve svalovině (0,155 $mg.kg^{-1}$) oproti játrům i ledvinám (tab. I a II). Poměr mezi zatížeností jaterní tkáně a svaloviny byl 6,90 : 1. Širší poměr byl mezi deponováním kadmia v ledvinách a kosterní svalovině (12,60 : 1).

U rtuti byla zjištěna průměrná koncentrace v játrech 0,132 $mg.kg^{-1}$, ledvinách 0,413 $mg.kg^{-1}$ a kosterní svalovině 0,020 $mg.kg^{-1}$. Poměr mezi zatížením svaloviny a jaterní tkáně činil 1 : 6,6. U svaloviny a ledvin byl zhruba dvojnásobný (1 : 13,9).

Nejvyšší průměrné koncentrace olova byly v ledvinách a játrech (tab. I a II). Podstatně nižší hodnoty byly ve svalovině (0,115 $mg.kg^{-1}$). Poměr mezi koncentrací ve svalovině a játry byl srovnatelný s poměrem mezi svalovinou a ledvinami (1 : 3,47 a 1 : 3,59).

Ze skupiny biogenních prvků byly sledovány koncentrace mědi a zinku. Zjištěné hodnoty obou prvků jsou uvedeny v tab. I až III. V souboru vyšetřených jedinců nebyly zaznamenány hodnoty koncentrace mědi a zinku, které by překročily hygienický limit (tab. IV). Poměr mezi koncentracemi mědi činil ve svalovině a játrech 1 : 2,09 a ve svalovině a ledvinách 1 : 2,05. Rovněž u zinku byly srovnatelné koncentrace mezi svalovinou a játry (1 : 1,60) i svalovinou a ledvinami (1 : 1,56).

V rámci histologických vyšetření byly zaznamenány reverzibilní a ireverzibilní změny různého rozsahu a charakteru. Výsledky ze sledované lokality jsou konfrontovány (tab. V) se závěry dlouhodobého monitoringu zdravotního stavu zaječí zvěře v ČR.

Na mozku převládaly drobné nespecifické změny. V hemisférách byly místy zjištěny kulatobuněčné perivaskulární infiltrace a mírný celulární edém. Vzácným nále-

zem byla inerfascikulární oligodendroglióza a tygrolýza pontu. Mozek byl celkově postižen u 20 % vyšetřených jedinců.

V plicích převládaly změny, které lze charakterizovat jako přímý důsledek odlovu (aktivní překrvení, edém, emfyzem). Pouze v jednom případě byla zjištěna výraznější katarální bronchopneumonie nejasné příčiny. Ojediněle byla diagnostikována verminozní pneumonie způsobená pneumohelminty rodu *Protostrongylus*.

Nejvíce postiženým orgánem byla játra (73,3 %). Zde převládala celá škála drobných nespecifických změn (výrazné lymfocytární infiltrace, binuklearní hepatocyty, hemosideróza Kupferových buněk, anisonukleóza) či počínajících degenerativních změn. Ojediněle se vyskytovala drobně kapénková forma steatózy, zřejmě jako důsledek působení škodlivých nox a dále velkokapénková forma steatózy, kterou lze klasifikovat jako morfologickou i funkční poruchu jater nutričního původu.

Na ledvinách byly charakteristické defekty typu drobných klínovitých jizev. Vyskytla se hemosideróza ledvin, častější byl záchyt degenerativních změn proximálních tubulů různého rozsahu.

Kromě drobných krvácenin se na slezině velmi často vyskytovala různě intenzivní hemosideróza. Tento orgán byl celkem postižen u 26 % vyšetřených vzorků zaječí zvěře.

Na gastrointestinálním traktu byla nejčastěji zastižena katarální enteritis. Relativně vysoký byl i výskyt čtyř druhů střevních kokcidií rodu *Eimeria* (86,6 %). Oocysty kokcidií se však zpravidla vyskytovaly ve slabé a střední intenzitě invaze.

Z reprodukčních orgánů nebyly na varlatech zajíců zaznamenány žádné podstatné změny. Naopak relativně hojné a pestré byly nálezy na vaječnících zaječek (25 %). Velmi často se vyskytovaly parovarialní cysty lokalizované zpravidla v hilu ovaria či cysty folikulární. Ojediněle byly zastiženy i zánětlivé změny či neprůchodnost vejcovodů.

zaječí zvěř; tkáně; chemické prvky; kontaminace; histologické defekty

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