

Micro- and macroelement contents in the liver of farm and wild animals and the health risks involved in liver consumption

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Abstract The paper presents the macroelement (Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn) and microelement (As, Cd, Co, Cr, Hg, Mo, Ni, Pb, and Sn) contents found in the liver of wild animals (boar and deer) and farm animals (rabbit, chicken, duck, cow, goat, and turkey). Statistically, the differences in element contents between the two groups were not significant (at $p = 0.05$), with the exception of Fe, K, Mg, Cd, Hg, Mo, and Pb. The liver of farm animals contained more Al, Cu, K, Mg, Na, Cr, and Sn, while the content of the remaining elements was higher in wild animals. An analysis of correlations between element content and age in wild animals (boar) showed that Pb and Al content increases with age, while Na and Cr contents decrease significantly. Comparisons between the test results and the maximum limits allowed by law showed that, in the case of wild animals, the regulatory limits were exceeded in 18% (for Cd and Cu) and 9% (for Hg) of the liver samples analyzed. In the case of farm animals, the limits for micro- and macroelement contents were not exceeded. The hazard index (HI) values for farm animals were lower than for wild animals, with regard to consumption by both children and adults. Based on the HI values calculated, it seems recommendable that consumption of the liver (preferably from farm animals) by children be limited to once weekly. For adults, the liver can be a valuable

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source of elements such as Zn, Fe, and Cr, which may be an indication for more frequent consumption.

Keywords Liver Wild and farm animals. Macroelements · Microelements · Health risk assessment

Introduction

The content of particular elements in various parts of the environment typically attracts much attention, and may even stir up emotion when viewed in the context of potential impact on living organisms, and especially on human health (Kicińska [2019](#page-17-0)). Health authorities pay particular attention to testing food produced by animal or plant farming for metal content. A number of research papers also discussed the issue of metal content in the tissues of wild animals (Bakowska et al. [2016,](#page-16-0) Dip et al. [2001](#page-16-0), Długaszek and Kopczyński [2011,](#page-16-0) Medvedev [1999](#page-17-0), Oyaro et al. [2007\)](#page-17-0). Wild animals are commonly believed to be healthier, and their meat is safer for consumption, due to their "healthier," more natural nutrition. Beside other factors, such as an animal's age, health, or sex, the kind of food the animal eats is a particularly important factor affecting the accumulation of toxins (e.g., metals) in tissue. Researchers, including Neila et al. ([2017\)](#page-17-0), Damek-Poprawa and Sawicka-Kapusta [\(2004\)](#page-16-0), Durkalec et al. [\(2015\)](#page-16-0), Antonkiewicz et al. [\(2018\)](#page-16-0), also demonstrated the importance of interspecies and individual differences resulting from adaptive processes related to inheritance of resistance in a given population or a given individual with increased

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resistance to high metal concentrations in the environment. Such resistance, involving the ability to exclude excess xenobiotics from metabolism or deposit them in specific organs, increases tolerance and changes the toxicity threshold (Kicińska [2016;](#page-17-0) Kicińska and Jelonek-Waliszewska [2017](#page-17-0); Kołacz et al. [2003](#page-17-0); Pietrzykowski et al. [2018](#page-17-0)). One such organ, especially with regard to metal accumulation, is the liver. It is a gland performing multiple functions (related to metabolism, circulation, and secretion), including the secretion of bile, which plays an important role in fat emulsification. The liver is involved in the synthesis and breakdown of glycogen; deamination and a range of amino acid transformations; synthesis of cholesterol, fatty acids, and phospholipids; and storage of blood and some vitamins and elements (e.g., Fe). It also contributes to the body's defense mechanisms by removing and breaking down harmful substances from the bloodstream (Damek-Poprawa and Sawicka-Kapusta [2004\)](#page-16-0).

The liver acts as a filter, or a chemical shield, that detoxifies the body by removing ingested toxins, physiological metabolites, or absorbed toxic chemicals. Its cells, called hepatocytes, absorb and deactivate dead, degenerated, or damaged cells that are no longer needed in the body, viruses and bacteria, and harmful substances ingested with food. These substances are neutralized by biochemical reactions and then excreted with bile. All kinds of the animal liver (chicken liver, cow liver, and others) are rich in minerals, including highly absorbable Fe, Cu, Zn, and Sn (Kabata-Pendias and Szteke [2012](#page-17-0)). It also has a high content of vitamins A, B2, B12, and C, folic acid, niacin, and pantothenic acid, greatly exceeding that found in any plant-based foods. Recommended values for human consumption vary depending on age and sex, ranging between 100 and 250 g of the animal liver per week (Burger [2007](#page-16-0); Szkoda and Żmudzki [2002\)](#page-17-0).

But is eating the liver really good for human health? We attempted to verify this by testing 33 liver samples from wild animals $(n = 11)$ and farm animals $(n = 22)$. The collected material was used to:

- a) determine the total content of the selected macroelements (Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn) and microelements (As, Cd, Co, Cr, Hg, Mo, Ni, Pb, Sn);
- b) identify differences in the content of these elements between wild and farm animals, as well as between the studied species (boar, deer, rabbit, chicken, cow, and goat);
- c) compare the test results with the regulatory limits for food and with data reported by other authors; and
- d) calculate the health risk involved in eating liver from both animal groups, associated with the content of selected elements (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, and Zn), for children and adults.

Material and area of research

Study material was collected between May and September 2017 in the Lesser Poland Province (southern Poland, EU). Fresh liver samples, 100 g or more per sample, were obtained at two livestock buying stations. At the same time, surveys were used to collect information on the animals' age, breeding and raising methods, and nutrition (for farm animals). For wild animals, the samples were obtained from three hunting clubs, and information on the animals' age, sex, and body weight was provided by the hunters.

Fresh, raw liver samples were placed in plastic bags and frozen until completion of the material collection stage. The total number of samples was 33, including 11 samples from wild animals (10 boars, including 6 males and 4 females, and 1 deer). The animals were aged between 6 months and 5 years. Most material was collected from farm animals ($n = 22$ samples), including 11 chickens (mostly aged 1–2 months), 4 rabbits (3– 12 months), 2 cows (2 and 18 months), 2 goats (2 and 36 months), 1 pig (6 months), 1 duck (8 months), and 1 turkey (18 months).

Study methods

Raw samples were frozen and stored in plastic bags at − 80 °C. Once all the samples had been collected, they were defrosted and subsequently dried at 50 °C for 24 h (Adrian and Stevens [1979\)](#page-16-0).

The dried material was ground using an electric grinder and treated with a mixture of concentrated acids $(65\%$ HNO₃ and 35–38% HCl, 1:3) at a 1:10 ratio of solids to solution. Additionally, mineralization was performed using a 30% H_2O_2 solution at 105 °C for 2 h. The solution was then cooled and poured into test tubes. The concentrations of Al, As, Ca, Cd, Co, Cr, Cu, Fe,

Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sn, and Zn in the extracts were determined by ICP-MS using an Elan 6100 system at the accredited AGH UST geohydrochemical laboratory in Kraków (certificate No. AB1050), with a precision of 10^{-5} mg/dm³. LOD and LOQ values were calculated using formulas presented in Kicińska [\(2018\)](#page-17-0) and are shown in Tables [1](#page-3-0) and [2.](#page-4-0) Repeatability of the results obtained was determined using an analysis of variance (FSP method—flexible statistical procedure—robust). Every 4th sample analyzed (the so called duplicate, their set comprised 25% of the population studied) was subject to distribution analysis and determination of element concentrations, just like the basic set of samples. The total variance and its components were as follows: $\delta^2_{total} = 0.121$, δ^2_{sam} . $_{\text{pling}} = 0.006, \delta^2_{\text{analysis}} = 0.002, \text{ and } \delta^2_{\text{ technical}} = 0.008.$ In accordance with the method used (Ramsey et al. [1992\)](#page-17-0), the obtained levels of uncertainty allow for making adequate chemical inferences. Study results were analyzed using the Statistica ver. 13.1 software.

To standardize the values published by other authors, we decided to report the test results in milligrams per kilogram of dry weight (DW), and fresh weight (FW) values reported in literature were converted to DW using formula (1) below, with an assumed moisture (water) content in the sample at 70%.

$$
C_{Me-md} = C_{Me-mw} \cdot \left(1 - \frac{x}{100}\right)^{-1},\tag{1}
$$

where:

 C_{Me} md content of the metal (Me) in a dry sample $(mg kg^{-1})$ C_{Me} mw content of the metal (Me) in a fresh sample $(mg kg^{-1})$ X moisture (water) content in the sample

To assess the potential health risk (HQ) for con-sumers, according to the US EPA methodology [\(2005,](#page-17-0) [2007](#page-17-0)), we used the determined concentration of selected elements found in the animal livers. Non-carcinogenic risk values were calculated using formula (2):

$$
HQ = \frac{EDI}{RfD},
$$
\n(2)

where:

- RfD reference dose according to US EPA ([2016](#page-17-0))
- EDI estimated daily intake (mg kg⁻¹ bw day⁻¹), calculated using formula (3):

$$
EDI = \frac{C \cdot EF \cdot EP \cdot F_{IR}}{W_{AB} \cdot T_A} \cdot CF1,
$$
\n(3)

where:

- C element content $(mg kg^{-1})$
- EF exposure frequency (365 or 52 days year⁻¹)
- ED exposure duration (70 years for adults, 6 for children)
- F_{IR} liver consumption (100 g person⁻¹ day⁻¹, calculated in mg)
- WAB average body weight (70 kg for adults, 20 kg for children) (US EPA [2011\)](#page-17-0)
- TA average exposure time (365∙ED), while in the case of carcinogens the value is 25,550 (days)
- CF1 unit conversion factor of 10^{-6}

Hazard index (HI) was calculated as the sum of HQ values. At $HI \leq 1$, the probability of adverse health effects is low, at $HI > 1$, adverse effects are probable, while values of $HI > 10$ indicate high exposure and high chronic health risk associated with toxicity. To establish a level of the cancer Risk for carcinogens, the HQ doses were multiplied by the respective slope factors (US EPA [2005](#page-17-0), for Pb WHO [1993\)](#page-17-0).

Results and discussion

Macroelement content in the liver of wild and farm animals

Out of all elements tested for, P and S dominated in the chemical composition, with values of 13,223– 20,216 mg kg⁻¹ DW (dry weight) for P and 8108– [1](#page-3-0)2,731 mg kg^{-1} DW for S (Table 1). Considerable levels of K, Na, and Al were also found, with contents of 4803–8076, 1259–3248, and 18–1277 (mg kg−¹ DW), respectively. As to highly absorbable elements, which included Fe, Ca, Cu, Mg, and Zn, the respective contents were 79–3144, 144–769, 4–139, 356–596, and 43–200 mg kg^{-1} DW. Small amounts of Mn were also found $(3–12 \text{ mg kg}^{-1} \text{DW})$.

A comparison of mean macroelement content found in the livers of wild and farm animals showed no significant differences (Fig. [1](#page-5-0)a–c). Samples from farm animals were found to contain more Al (by 21% on average), Cu (16%), and K, Mg, and Na (10%). Mn and P levels were found to be nearly identical in these two animal groups. Slightly lower results were obtained

*Statistical significant for $p < 0.05$

*Statistical significant for $p < 0.05$

 n no. of samples, SD standard deviation, V variability coefficient, $-$ lack data

 1 PL—permitted level (for wet weight, in bracket value transformed to dry weight) according to Regulation [\(2003\)](#page-17-0)

*Statistical significant for $p < 0.05$

for farm animals than for wild animals with regard to content of Zn (23% lower) and Ca and Fe (approx. 9% lower). However, in the samples from farm animals, the dispersion of values was considerably larger (Fig. [1\)](#page-5-0). The differences in macroelement content between wild and farm animal liver were not statistically significant (at $p = 0.05$), except in the case of Fe, K, and Mg. In the studied population of wild animals, the respective levels of these elements were 448, 6141, and 1761 mg kg^{-1} DW, while in the population of farm animals, the respective values were 408, 6731, and 498 mg kg⁻¹ DW (Table [1\)](#page-3-0).

Microelement content in the liver of wild and farm animals

The microelement group included, As, Cd, Co, Cr, Hg, Mo, Ni, Pb, and Sn, and the content of these elements in the samples was 0.04–0.56, 0.03–4.02, 0.01–0.12, 2.34–8.07, 0.01–0.40, 1.2–4.61, 1.81–3.09, 0.08–1.01, and 1.13–1.77 mg kg^{-1} DW, respectively (Table 2). For wild animals, the mean levels were as follows (in mg kg−¹ DW): As 0.16, Cd 1.15, Co 0.07, Cr 2.81, Hg 0.07, Mo 2.67, Ni 2.29, Pb 0.45, and Sn 1.35. For farm animals, the calculated means were slightly higher in two cases (values in mg kg⁻¹ DW): for Cr the mean was 2.99 (i.e., approx. 6% higher than for wild animals), and for Sn, it was 1.44 (approx. 7% higher). The mean levels of the remaining elements were between 4 and 70% lower than in wild animals. The values (in mg kg^{-1}) DW) were as follows: As 0.10, Cd 0.34, Co 0.06, Hg 0.02, Mo 2.04, Ni 2.09, and Pb 0.21 (Fig. [1d](#page-5-0), e). Statistically significant differences (at $p = 0.05$) were only found for Cd, Hg, Mo, and Pb.

The highest variability in the entire material was found for the concentrations of Hg (V = 30), Cu (V = 26), Fe (V = 22), Al (V = 21), and Cd (V = 20). In this group, the so-called extreme values were also found (Fig. [1\)](#page-5-0).

Species-, age-, and sex-related differences in chemical composition

Though the studied animal populations were not large, an attempt was made to analyze differences in the

Fig. 1 Statistical diversity of content of macro- (a-c) and microelements (d-e) in the livers of wild (W) and farmed (F) animals

chemical composition of the liver in terms of the species, age, and sex of the animals.

Wild animals

In the wild animal group, the boar liver contained more Al, Ca, Cd, Co, Cr, Fe, Ni, Pb, and Zn than the deer liver. The mean levels of these elements were 224.3, 465.4, 1.17, 0.06, 2.82, 480, 2.32, 0.49, and 95.3 mg kg^{-1} DW, respectively, in the boar liver, and 208.8, 430.8, 0.91, 2.75, 129, 1.95, 0.08, and 49.11 mg kg^{-1} DW, respectively, in the deer liver. The deer liver contained more As $(0.92 \text{ mg kg}^{-1} \text{DW})$, Co $(0.10 \text{ mg kg}^{-1} \text{DW})$, and Cu $(61.60 \text{ mg kg}^{-1} \text{DW})$ —in the boar liver, the levels of these elements were 0.16, 0.06, and 14.48 mg kg−¹ DW, respectively. Mg and Mn contents were similar between the two species (Table [3\)](#page-6-0).

Comparisons between the obtained results and values reported by other authors show that:

– the obtained results for Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, and Zn levels are considerably higher than those reported by Długaszek and Kopczyński ([2011\)](#page-16-0) in Polish boars, and the obtained As and Al levels are considerably higher than those reported by Kucharczak and Moryl ([2012](#page-17-0)) in the same population;

- the obtained Cd levels are slightly higher than those reported by Medvedev [\(1999](#page-17-0)), who studied the livers of Russian boars, while Pb results reported by the author were very similar to those found in the present study;
- the obtained results for Cd, Cu, Pb, and Zn were similar to those reported by Neila et al. ([2017](#page-17-0)) in the boar liver. For Cd, Pb, and Zn, the mean levels reported by the author were 0.33, 0.38, and 56.86 mg kg^{-1} DW, respectively—slightly lower than those found in the present study: 1.17, 0.49, and 95.3 mg kg^{-1} DW, respectively. For Cu, the opposite was found in the Spanish samples analyzed by Neila et al. ([2017](#page-17-0)); the mean content of this metal was 23.50 mg kg^{-1} DW (range 9.16–63.26 mg kg⁻¹ DW), while in the present study, the mean Cu content was 14.48 mg⋅kg⁻¹ DW, and the range was 8.03– 43.95 mg kg^{-1} DW; and
- with regard to the deer liver, we found considerably higher levels of Al, As, Ca, Cr, Mn, and Ni; comparable levels of Cd, Co, and Mn; and considerably lower levels of Cu, Fe, Mg, Pb, and Zn than those reported for the deer liver samples by Khan et al. [\(1995](#page-17-0)) and Długaszek and Kopczyński ([2011](#page-16-0)) (Table [3](#page-6-0)).

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Khan et al. [\(1995](#page-17-0))/USA (wet weight calculated to dry weight)

⁴ Kucharczak and Moryl (2012)/Poland (wet weight calculated to dry weight) Kucharczak and Moryl [\(2012](#page-17-0))/Poland (wet weight calculated to dry weight) ⁵ Długaszek and Kopczyński (2011)/Poland (wet weight calculated to dry weight) Długaszek and Kopczyński [\(2011](#page-16-0))/Poland (wet weight calculated to dry weight)

 6 This study (2017)/Poland (dry weight) This study (2017)/Poland (dry weight)

In the boar population, samples were collected both from males and females. The calculated means and standard deviations indicate that the levels of Al, As, Hg, K, Mg, Pb, and Zn are higher in male boars (Table 4). The livers of female boars contained approx. 5–10% less Al, K, and Mg and approx. 30–40% less As, Pb, Zn, and Hg. On the other hand, the livers of female boars contained approx. 5–7% more Cr, Na, and Sn; approx. 10–20% more Ca, Fe, Mo, Ni, Mn, and S; approx. 40% more Co; and over 100% more Cu and 150% more Cd. P concentrations were nearly identical in both sexes. Very high variability was found for Al and Hg in males and for Cd and Cu in females. Similar findings were reported by Khan et al. ([1995](#page-17-0)) in their analysis of levels of Cd and other heavy metals in the liver of white-tailed deer in Alabama. They found higher Cu and Zn levels in females and higher Cd, Co, and Ni levels in males. In a study of boars living in Galicia (NW Spain), Neila et al. ([2017\)](#page-17-0) found higher Zn, Cd, and Pb

Table 4 Average contents (mg kg^{-1} dry weight) and standard deviations of elements calculated for the livers taken from female and male of wild boar species

Element	Arithmetic mean \pm standard deviation	
	Male specimens $(n=6)$	Female specimens $(n=4)$
A1	229.3 ± 85.5	216.9 ± 30.5
As	0.18 ± 0.07	0.13 ± 0.01
Ca	425.9 ± 22.2	524.5 ± 56.7
C _d	0.74 ± 0.13	1.81 ± 0.67
Co	0.05 ± 0.006	0.08 ± 0.02
Cr	2.74 ± 0.09	2.93 ± 0.02
Cu	10.27 ± 0.80	20.79 ± 7.11
Fe	435.1 ± 71.6	546.9 ± 74.0
Hg	0.09 ± 0.05	0.06 ± 0.02
K	6311 ± 160	5822 ± 133
Mg	457.5 ± 18.7	442.9 ± 12.5
Mn	6.80 ± 0.9	7.90 ± 0.51
Mo	2.47 ± 0.13	2.87 ± 0.63
Na	1756 ± 174	1841 ± 81
Ni	2.22 ± 0.11	2.48 ± 0.22
P	$16,066 \pm 443$	$16,054 \pm 743$
Pb	0.56 ± 0.14	0.39 ± 0.06
S	9930 ± 426	$10,787 \pm 576$
Sn	1.32 ± 0.04	1.40 ± 0.09
Zn	111.4 ± 16.6	71.1 ± 10.4

 n number of sample

contents in the liver samples from males (55.78, 0.346, and 0.424 mg kg^{-1} DW, respectively), compared to samples from females (45.25, 0.305, and 0.341 mg kg^{-1} DW, respectively). The opposite was found for Cu, with slightly higher concentration of this metal found in female boars (23.83 mg kg⁻¹ DW) than in males (23.08 mg kg⁻¹ DW).

With a significance threshold set at $p = 0.05$, the calculated differences in levels of the analyzed elements between the livers from male and female boars were not significant.

Correlation coefficients were calculated for element content and animal age (at three significance thresholds: $p = 0.05$, $p = 0.01$, and $p = 0.001$) in the wild animal population (Table [5\)](#page-8-0). High correlation $(0.5 \le r < 0.7)$ was found for the following combinations: Mg–K, Mg–S, S–Mn, Fe–Cd, Cd–P, Co–Cu, Cr–Al, Cr–Na, Ni–Na, Pb–Na, Pb–As, and Pb–Hg. Similarly high inverse correlation was found for Na–Al, Cu–Fe, Zn–Cu, and Pb–Al. Very high correlation $(0.7 \le r < 0.9)$ was found for P–Mg, Mn–Al, Zn–K, Mo–Cd, and Ni–Ca. Almost full correlation $(0.9 \le r < 1)$ was only found for the Hg–As pair.

The following correlations were found to be statistically significant: S–P, Cd–S, and Mo–P at $p = 0.05$; P– Mg, Mn–Al, Zn–K, Mo–Cd, and Ni–Fe at $p = 0.01$; and Hg–As at $p = 0.001$.

An analysis of correlations between element content and age in wild animals showed that Pb $(r = 0.57)$ and Al ($r = 0.62$) contents increase with age, while Na ($r = -$ 0.84) and Cr $(r = -0.65)$ contents decrease significantly. For the remaining elements, no statistically significant correlations were found. Similar findings were reported by Khan et al. [\(1995\)](#page-17-0), who found a statistically significant correlation between age and Ni content only and no significant correlation (at $p < 0.05$) for Cd, Co, Cu, and Zn.

Farm animals

The other, much larger, group studied included farm animals, and specifically, chicken, duck, goat, turkey, rabbit, and cow (calf). Comparisons for such a large number of elements are rather difficult, mainly due to the wide variance in their concentrations. Therefore, the analyzed elements were divided into four groups (Fig. [2\)](#page-10-0). Group 1 included P, S, K, and Na (Fig. [2](#page-10-0)a). Their presence in animal tissue is obvious, and these were the dominant elements out of all analyzed in the

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* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ statistically significant

present study (Table [1\)](#page-3-0). The highest P content was found in the goat liver (mean 18,393 mg kg⁻¹ DW), with slightly lower values in the pig (16,807 mg kg⁻¹ DW) and chicken livers (16,702 mg kg⁻¹ DW). The lowest P content was found in the rooster liver (13,223 mg kg⁻¹ DW). These results are considerably higher than the P content listed in the basic report from USDA ([ndb.nal.](http://ndb.nal.usda.gov) [usda.gov\)](http://ndb.nal.usda.gov). For S, the highest levels were found in the duck liver (11,697 mg kg⁻¹ DW), the lowest in the rabbit liver (9698 mg kg^{-1} DW) which also contained the least K (4,803 mg kg⁻¹ DW). The highest K content was found in the chicken liver (7214 mg kg⁻¹ DW). Na levels were the highest (2709 mg kg^{-1} DW) in the rooster liver and the lowest (1506 mg kg⁻¹ DW) in the cow liver.

Group 2 included the elements required for normal growth and functioning of the animal body: Ca, Fe, Al, Zn, and Cu (Fig. [2b](#page-10-0)). The highest levels of these elements were found (respectively, in mg kg^{-1} DW) in the following: rabbit (495), turkey (3144!!), cow (534), pig (164), and goat livers (64). The lowest Ca, Fe, Al, Zn, and Cu levels were found (respectively, in mg kg^{-1}) in the following: duck (370), cow (89), turkey (18), rooster (54), and chicken livers (8.6). The Ca values found in the duck, pig, cow, and chicken livers are considerably higher than those published by the USDA [\(ndb.nal.](http://ndb.nal.usda.gov) [usda.gov\)](http://ndb.nal.usda.gov). The Cu levels found in the present study are considerably higher than those reported by Oyaro et al. ([2007](#page-17-0)) in Kenyan cattle, but very similar to those found by Kołacz et al. [\(2003\)](#page-17-0) in chickens form Polish farms (Table [6\)](#page-11-0). A significant difference was found for Fe. We found a mean Fe level of 89 mg kg^{-1} DW in the cow liver, whereas the US agency published a value of 65 mg kg⁻¹ FW or 218 mg kg⁻¹ after conversion to DW. Much smaller differences were found for the chicken liver: in the present study, we found a mean Fe content of 240 mg kg−¹ DW in these samples, while the USDA reports a value of 116 mg kg⁻¹ FW or 387 mg kg⁻¹ DW. The Zn levels we found in the duck and pig livers were nearly identical to those reported in the database available at [ndb.nal.usda.gov.](http://ndb.nal.usda.gov) For the cow and chicken livers, our results were lower [\(ndb.nal.usda.gov,](http://ndb.nal.usda.gov) Kołacz et al. [2003](#page-17-0)).

Group 3 included elements found in concentrations of several ppm: Mn, Cr, Mo, Ni, and Sn (Fig. [2c](#page-10-0)). Some of these elements (e.g., Mn, Cr) are involved in carbohydrate or calcium metabolism; others (e.g., Mo, Sn) play a role in oxidation–reduction processes. The highest mean Mn content was found in the turkey liver $(11.85 \text{ mg kg}^{-1} \text{DW})$; Cr and Sn—in the chicken liver (3.39 and 1.53 mg kg⁻¹ DW, respectively); Mo—in the pig liver (2.78 mg kg⁻¹ DW); and Ni—in the goat liver $(2.21 \text{ mg kg}^{-1} \text{DW})$. The lowest mean Mn, Cr, Mo, Ni, and Sn contents were found (respectively, in mg kg^{-1} DW) in the following: chicken (6.42), duck (2.48), rooster (1.45) , turkey (1.90) , and pig livers (1.23) .

Group 4 included those microelements that are considered as having no benefit with regard to growth and development: Cd, Pb, As, Hg, and Co. In high concentrations, these elements can be highly toxic or even carcinogenic. The highest Cd, Pb, As, Hg, and Co contents were found (respectively, in mg kg^{-1} DW) in the following: rooster (0.896), duck (0.650), pig (0.163), and turkey livers (0.051 and 0.100) (Fig. [2d](#page-10-0)). The lowest values were found (respectively, in mg⋅kg⁻¹ DW) in the following: chicken (0.191), duck (0.125), rooster (0.043), turkey (0.018), and pig livers (0.033).

These results were higher than those reported by Szkoda and Żmudzki ([2002\)](#page-17-0) for pigs and rabbits (Table [6](#page-11-0)). The present Cd levels were lower than those reported by Kołacz et al. ([2003](#page-17-0)) in their analysis of the chicken liver. The Pb content in the pig, cow, chicken, and rabbit livers found in the present study was similar to that reported by Szkoda and Żmudzki ([2002](#page-17-0)), while that found in the duck liver was higher (Table [6\)](#page-11-0). With regard to As and Hg, Szkoda and Żmudzki ([2002](#page-17-0)) found considerably lower values for the pig, cow, rabbit, and chicken livers (approx. two to five times lower).

In the studied farm animal population, statistical analyses of age-related differences in element content would be pointless, as the groups were small and did not differ greatly in terms of age.

Health risk involved in consumption of the animal liver

Consumption of food not only supplies the necessary energy but also fulfills the body's demand for macroand microelements. The WHO publishes the recommended intakes of these substances, which ensure healthy development while preventing any toxic effects resulting from excesses or deficiencies. National legislations also contain recommendations on the allowed content of selected elements (mainly trace elements) in a variety of food products (including the liver). Thus, the Polish Health Ministry Regulation of 2003 and the Commission Regulation (EC No. 1881/[2006](#page-16-0)) stipulate the maximum allowed content of particularly harmful elements in the animal liver, which amounts to

Fig. 2 Average content of macro- (a-b) and microelements (c-d) in the livers of farmed animals

0.50 mg kg⁻¹ FW for As, Cd, and Pb, and 0.05 mg kg⁻¹ FW for Hg. Comparisons between the test results and the maximum content limits showed that, in the case of wild animals, the regulatory limits were exceeded in 18% (for Cd and Cu) and 9% (for Hg) of the liver samples analyzed. The results for farm animal liver samples are much better, as none of these elements exceeded the allowed limits (Table [2\)](#page-4-0). Among macroelements, maximum content is only regulated for Cu and Zn and amounts to 10 and 150 mg kg^{-1} FW, respectively. Based on the obtained results, 18% of wild animals and 9% of farm animals have excessive levels of Cu, but all samples were within the regulatory limits for Zn.

RDI

For the purpose of assessing the health risk involved in human consumption of animal products, we used 100 g as the daily animal liver consumption, based on the FAO/WHO expert committee report on veterinary drug residues (EC No. 782/[2018](#page-16-0)). Provisional Tolerable Weekly Intake (PTWI) and other selected intake indicators (including ADI, MRL, RDI, TDI—abbreviations are explained below Table [7\)](#page-14-0) for the selected macro- and microelements based on FAO/WHO recommendations are shown in Table [7.](#page-14-0) The table also includes the RfD (reference dose) values used in HQ calculations (see "[Study methods](#page-1-0)," formula [2](#page-2-0) above). The collected data were used to propose a Recommended Daily Intake (RDI) value for each of the elements analyzed. A comparison between the calculated RDI for children (BW = 20 kg) and adults (BW = 70 kg) and the levels of selected elements found in the animal liver (converted to fresh weight using formula [1\)](#page-2-0) demonstrated that:

1. In the samples from wild animals, the calculated RDI values for children were exceeded in one sample with regard to Pb, Hg, As, and Zn; in three samples with regard to Cu; in four---P; in five--Al; in six—Cd, and in seven—Fe. The levels of other elements, i.e., Zn, Co, Ni, and Mo, did not exceed the RDI.

Results for farm animal liver samples $(n = 22)$ were somewhat better. RDI values were not exceeded for Sn, Co, Ni, Mo, Pb, Hg, As, or Zn. Cd content exceeded the limit in just 1 sample, Fe in four samples, Cu—five, Al—eight, and P—nine samples.

2. The RDI values for adults were not exceeded in samples from farm and wild animals with regard to: Ca, K, Hg, Mg, Mo, Na, P, Ni, Mn, Zn, As, Co,

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– no data

¹ This study (2017)/Poland (dry weight) This study (2017)/Poland (dry weight)

 2 Data from https://ndb.nal.usda.gov/; (wet weight calculated to dry weight) Data from <https://ndb.nal.usda.gov/>; (wet weight calculated to dry weight)

³ Kołacz et al. (2003)/Poland (wet weight calculated to dry weight) Kołacz et al. ([2003](#page-17-0))/Poland (wet weight calculated to dry weight)

4 Oyaro et al. (2007)/Nairobi, Kenya (dry weight) Oyaro et al. ([2007](#page-17-0))/Nairobi, Kenya (dry weight)

⁵ Sikora (1994)/Poland (wet weight calculated to dry weight) Sikora ([1994](#page-17-0))/Poland (wet weight calculated to dry weight)

⁶ Szkoda and Żmudzki (2002)/Poland (wet weight calculated to dry weight) Szkoda and Żmudzki ([2002](#page-17-0))/Poland (wet weight calculated to dry weight)

and Sn. In one sample from a wild animal (boar), the RDI for Pb and Cd was exceeded. The RDI for Cu was exceeded in two liver samples, boar and deer, and the RDI for Fe was exceeded in seven boar liver samples. In the farm animal liver samples, RDI for Cd was exceeded in 1 case (turkey), Al—in three cases (cow, rabbit, chicken), Cu—in three (goat, duck, rabbit), and Fe—in four (got, duck, pig, turkey).

3. Cr levels were the most alarming, as they exceeded the RDI both for children and for adults in all the analyzed liver samples, collected from wild as well as farm animals.

HQ

Non-carcinogenic health risk was analyzed for the following elements: Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, and Zn, due to the availability of RfD values that could be used in hazard quotient (HQ_{ino}) calculation. HQ_{ing} was calculated for children and adults with an assumed $F_{IR} = 100$ g (Table [8\)](#page-15-0).

For children and adults eating liver from wild animals, either once daily or once weekly, the following sequence of HQ_{ing} values was obtained, in descending order:

$$
Fe > Cd > Cr > As > Mo > Cu > Zn > Al > Hg
$$

$$
> Mn > Pb > Ni > Co.
$$

With regard to the liver from farm animals, the descending HQ_{ing} value sequence for children and adults was different:

 $Fe > Cr > Cu > Mo > As = Cd > Al > Zn > Mn$ $>$ Hg $>$ Ni $>$ Pb $>$ Co.

The differences between the two sequences above result from different content of particular metals (especially Cd and Cu) in the two sample groups.

Based on the specific HQing values calculated for children, we found HQ_{ing} values > 1, which indicate a possible health risk, only for daily consumption of the liver from wild animals, due to Fe (2.23E+00), Cd $(1.75E+00)$, and Cr $(1.40E+00)$ content. With the farm animal liver, the only risks were related to Fe (2.03E+ 00) and Cr (1.50E+00) content. With once per week consumption of the liver, the HQ_{ing} values for all the analyzed elements were below 1, which indicates no health risk. For adults, all HQ_{ing} values were below 1, both for daily and weekly consumption of the liver and for wild and farm animals. This warrants the conclusion that the product does not pose a health risk.

While the high Fe content in the animal liver is not problematic (indicating instead that the liver is an important source of highly absorbable Fe). Iron overload may occur but only when the element is delivered in supplements. With iron delivered in food, the organism carefully regulates its levels, assimilating this microelement according to the current needs. Any excess of this element is safely removed (it is not assimilated). The most frequent reason for elevated iron levels is a genetic predisposition for accumulation of iron, i.e., hemochromatosis. It is usually caused by a mutation of the HFE gene. The health risk associated with high Cd and Cr content can be alarming.

Cd accumulates in the human body throughout the life, due to the element's long half-life (10–33 years). Kabata-Pendias and Szteke ([2012](#page-17-0)) state that up to 15% of adsorbed Cd can accumulate in the liver. The metal inhibits phosphatase and supersedes Zn, which is required for healthy function of the body. Excess Cd impairs kidney function, Ca metabolism, and vitamin B12 transformation, leading to anemia and hypertension. The accumulation of Cd is particularly harmful in young individuals, causing irreversible alterations to the central nervous system. This is why the element is considered a group 1 carcinogen.

Cr was another element with a $HQ_{ing} > 1$. It is needed for human and animal health, and has two forms: Cr^{3+} and Cr^{6+} . Cr plays an important role in protein and lipid metabolism, as well as in the action of insulin. In food, it is mainly present as Cr^{3+} , though this does not apply to the liver. Cr content in the body decreases with age, and the decrease is particularly marked in the liver. Therefore, the element is often included in the so-called geriatric supplements.

Hazard and risk indexes

The final hazard index (HI) values, which are the sum of all HQ_{ing} values calculated for children and adults, considering daily or weekly consumption, are shown in Table [8](#page-15-0). The HI values for children are approx. 3.5 times higher on average than HI values for adults. The HI values for children and adults with the same exposure

Table 7 Recommended Daily Intake (RDI) for children and adults of macro- and microelements

N.S. not specified, RDI Recommended Daily Intake, TDI Tolerable Daily Intake, ADI Acceptable Daily Intake, PTWI Provisional Tolerable Weekly Intake, PTMI Provisional Tolerable Monthly Intake, PTMI Provisional Tolerable Monthly Intake

 $1 \text{http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID = 3511$ $1 \text{http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID = 3511$ $1 \text{http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID = 3511$

2Recommendation from TRS 776-JECFA 33/27 ([1988](#page-17-0))

³ Recommendation from TRS 930-JECFA 64/26 ([2005](#page-17-0))

⁴ Kabata-Pendias and Szteke ([2012](#page-17-0)) data for adults

⁵ MRL—minimal risk levels, data from <https://www.atsdr.cdc.gov>

⁶ Data from <https://cfpub.epa.gov/ncea/iris2> for oral exposure

⁷ Data from IŻŻ—National Food and Nutrition Institute (Poland)

frequency, but different sources of the liver (farm or wild animals), differ by approx. 30%, which is due to the different element content in the liver of wild and farm animals.

Italicized values: HQ_{ing} , $HI > 1$

The HI for children (with daily or weekly consumption of the liver from wild or farm animals) and for adults (only with daily consumption, but either source of the liver) is greater than 1, which may indicate a possible adverse health impact. No such risk was found for adults eating liver from wild or farm animals once weekly (Table 8).

The HI values for element content in the farm animal liver were lower than those for the wild animal liver, with regard to consumption by both children and adults. In the context of the analyzed macro- and microelement contents, this may indicate that the farm animal liver is "safer" for human consumption.

With daily animal liver consumption in the case of children, the risk index was 3.21E−05 and 1.93E−05 for wild and farm animals, respectively. These values are close to the limit considered safe (10E−05). As for the scenario assuming animal liver consumption once weekly and once daily in the case of adults, the risk index ranged between 7.85E−07 and 9.18E−06. Since these values are below the permissible limit, this situation can be deemed satisfactory.

Conclusions

Based on our analysis of macro- and microelement contents in 33 liver samples collected from wild animals (boar, deer) and farm animals (chicken, cow, duck, turkey, goat), the following conclusions can be formulated:

- 1. No significant differences were found in the macroelement levels analyzed (Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn) between the livers from wild and farm animals. The farm animal liver contained more Al, Cu, K, Mg, and Na (the difference ranged between 9 and 23%). Mn and P levels were found to be nearly identical in these two animal groups. Slightly lower results were obtained for farm animals than for wild animals with regard to content of Zn, Ca, and Fe.
- 2. With regard to microelements (As, Cd, Co, Cr, Hg, Mo, Ni, Pb, and Sn), farm animal liver had a higher mean content of Cr and Sn only. The mean levels of the remaining elements were between 4 and 70% lower than in wild animals.
- 3. Statistically, the differences in element contents between the livers from wild and farm animals were not significant (at $p = 0.05$), with the exception of Fe, K, Mg, Cd, Hg, Mo, and Pb.
- 4. In the samples from farm animals, there was a considerably larger dispersion of macroelement levels, and the highest variability in the entire material was found for the concentrations of: Hg, Cu, Fe, Al, and Cd.
- 5. The liver of female boars contained less Al, K, Mg, As, Pb, Zn, and Hg but more Cr, Na, Sn, Ca, Fe, Mo, Ni, Mn, S, Co, Cu, and Cd. P concentrations were nearly identical in both sexes. Very high variability was found for Al and Hg in males and for Cd and Cu in females. With a significance threshold set at $p = 0.05$, the calculated differences in levels of the analyzed elements between the livers from male and female boars were not significant.
- 6. An analysis of correlations between element content and age in wild animals showed that Pb and Al contents increase with age, while Na and Cr contents decrease significantly. For the remaining elements, no statistically significant correlations were found.
- 7. Comparisons between the test results and the maximum limits allowed by law showed that, in the case of wild animals, the regulatory limits were exceeded in 18% (for Cd and Cu) and 9% (for Hg) of the liver samples analyzed. The results for farm animal liver samples are much better, as none of these elements exceeded the allowed limits.
- 8. The HI values for the liver from farm animals were lower than for wild animals, with regard to consumption by both children and adults. In the context of the analyzed macro- and microelement contents, this may indicate that the farm animal liver is "safer" for human consumption.
- 9. Based on the HI values calculated, it seems recommendable that consumption of the liver (preferably from farm animals) by children be limited to once weekly.
- 10. For adults, the liver can be a valuable source of elements such as Zn, Fe, and Cr, which may warrant more frequent consumption.

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