Journal of Microbiology, Biotechnology and Food Sciences

Binkowski et al. 2012 : 2 (1) 304-313

REGULAR ARTICLE

XENOBIOTIC METALS IN MUSCULES AND BONES OF MALLARD FROM SOUTHERN POLAND

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ABSTRACT

Samples collected from adult males of Mallard (shot in 2011 near Zator) were analyzed in the aspect of cadmium and mercury concentrations. Among bones samples of radius, ulna, humerus, femur, tibia, crista sterni and rostrum were taken. From muscles musculus pectoralis major, m. pectoralis minor, m. gastrocnemius, m. tibialis anterior, m. biceps, m. triceps, m. external oblique, and m. lingua were sampled. Concentrations were measured with AA spectrometer and mercury analyzer. Cadmium concentrations were lower in muscles – only 7% of these samples have concentrations higher than the detection limit. Cadmium content in bones are not high (medians range 1.962 – 2.508 µg/g d.w.). Mercury concentrations in bones were diverse: from 0.016 (ulna) to 0.213 (rostrum) µg/g w.w. In muscles accumulation was low and medians fitted in the range between 0.020 (biceps) and 0.025 µg/g w.w.

Keywords: cadmium, mercury, waterfowl, accumulation, food, meat

INTRODUCTION

Water areas are places under a high anthropogenic pressure. Next to the air pollution also water and especially sediments can be sources of numerous xenobiotics **(Kabata-**

Pendias and Pendias, 1999; Babińska *et al*., 2008). One from the widest group of these substances are heavy metals. Three of them are known as highly toxic – cadmium, mercury and lead **(Scheuhammer, 1987)**. All mentioned metals are strictly connected with anthropogenic activity what confirmed high values of anthropogenic enrichment factors **(Walker *et al*., 2006)**. The phenomenon of lead poisoning among waterfowl was noted in many different places of the world **(Pain, 1990; Scheuhammer and Norris, 1995; Binkowski and Zakrzewska, 2009; Binkowski, 2011)**. Data concerning concentrations of cadmium and especially mercury are not so numerous, but the wage of these problems is also very significant.

One from the most visible and biodiverse group of animals living on water areas are birds. Mallard is the commonest duck in the world **(Cramp, 1998)**. This is a popular game bird **(MŚ, 2005)** whose meat is commonly consumed, mostly beyond the official market by hunters. Carcasses are not demanded to be researched in the aspect of parasites and even chosen xenobiotics. As we know such food can be a significant source of xenobiotics (including metals) for people – game consumers **(Johansen *et al*., 2004; Mateo *et al*., 2007; Binkowski, 2012a)**.

The main goal of this work was to assess the amount of cadmium and mercury in bones and muscles of Mallard. These parts of the carcass are used as ingredients of game food in many countries. Determined values were compared to official law demands which were laid out for poultry admitted to the market.

MATERIAL AND METHODS

Research was carried out on eleven adult males of Mallard *Anas platyrhynchos* L. All birds were shot with lead pellets (No. 4) during the hunting on 27th August 2011 on Przeręb fishponds near Zator in southern Poland. After the shot, birds were weighed and frozen (-18°C).

After a defrosting in the laboratory (Institute of Biology, Pedagogical University of Cracow) samples of bones and muscles were taken. Among bones following samples were collected: radius, ulna, humerus, femur, tibia, crista sterni and rostrum (beak is a corneous sample but we treat it as a bone). In the case of muscles tissues were sampled as follows: *musculus pectoralis major*, *m. pectoralis minor*, *m. gastrocnemius*, *m. tibialis anterior*, *m. biceps*, *m. triceps*, *m. external oblique*, and *m. lingua*. Taken samples were put on glass Petri dishes, weighed (0.0001 g, Radwag WPA 60/K) and put into the laboratory dryer (60°C). The dry weight obtained according to method described by **Binkowski (2012b)** was used in the further protocol.

Dry samples were mineralized (Velp Scientifica mineralizer DK20/26) in the hot mixture of nitric (65%, Baker Analyzed) and perchloric (70%, Baker Analyzed) acids. Such prepared solutions, fulfilled up to 10 mL with deionized water (18.2 µS) were analyzed with flame atomic absorption spectrometer (Buck Scientific 200 AAS) at the wavelength of 228.8 nm. The limit of detection (LoD) for cadmium was on the level of 0.031 mg/L. Cadmium concentrations are given in µg/g of the dry weight (µg/g d.w.).

Mercury was analyzed in wet weight of samples of five drakes (randomly selected from collected specimens) with MA-2000 mercury analyzer (Nippon Instruments Corporation). Absolute limit of detection in sample was 0.002 ng. Concentrations of mercury are given in µg/g of the wet weight (µg/g w.w.).

Statistical analysis was done with Statistica 10 (StatSoft). Because the data did not meet the parametric tests' demand (normal distribution - Shapiro Wilk test and homogeneity of variance – Levene test), Kruskal-Wallis test was carried out (significance level 0.05). Also Spearman correlation factor was calculated to assess possible correlations in metals concentrations in different bones and muscles.

RESULTS AND DISCUSSION

Among bones crista sterni was the most hydrated tissue (70.06% of d.w.). Bone which accumulated the smallest amount of water was humerus (91.15% of d.w.) (Table 1). Differences among these values were statistically significant (Kruskal-Wallis test, P<0.0001).

Table 1 % of d.w. (medians) of collected samples (n=11)

Bone	% d.w.	Muscle	% d.w.
radius	86.95[a]	pectoralis major	27.43[a]
ulna	88.64[a]	pectoralis minor	26.35[a]
humerus	91.15[a]	gastrocnemius	26.83[a]
femur	82.25[ab]	tibialis anterior	25.92[a]
tibia	85.90[a]	biceps	27.72[ab]
crista sterni	70.06[b]	triceps	25.58[a]
rostrum	74.54[b]	external oblique	27.44[a]
		lingua	39.60[b]

Legend: [a,b] different letters stress statistically significant differences

Also significant differences in water content in tissue occurred among muscles (Kruskal-Wallis test, $P<0.0001$). The highest content of water was noted in triceps, the smallest amount in lingua muscle (Table 3).

Cadmium

Median values of cadmium concentrations in all muscles and bones (except radius) were lower than the limit of detection of the used procedure (Tab 2). The highest concentration of this element among bones occurred also in femur (4.072 µg/g d.w.) and among muscles in musculus gastrocnemius (1.482 µg/g d.w.). Differences in concentrations of cadmium were statistically significant only in the case of bones (Kruskal-Wallis test, $P=0.0145$).

Table 2 Cadmium concentrations (µg/g d.w.) in bones and muscles of Mallard (n=11)

Bone	Minimum	Maximum	Median	Median	Maximum	Minimum	Muscle
ulna	< LoD	2.608	2.159^{ab}	< LoD	1.413	< LoD	pectoralis major
radius	1.692	3.358	2.508^{ab}	< LoD	1.045	< LoD	pectoralis minor
humerus	< LoD	2.916	2.279^{ab}	< LoD	1.482	< LoD	gastrocnemius
femur	< LoD	4.072	2.738^{a}	< LoD	< LoD	< LoD	tibialis anterior
tibia	< LoD	3.222	2.493^{ab}	< LoD	< LoD	< LoD	biceps
crista sterni	< LoD	2.794	2.368^{ab}	< LoD	< LoD	< LoD	triceps
rostrum	< LoD	3.032	1.962^{b}	< LoD	1.239	< LoD	external oblique
				< LoD	0.716	< LoD	lingua

Legend: ^{a,b} different letters stress statistically significant differences

No significant correlations in cadmium concentrations among bones and among muscles were found.

Mercury

The highest concentrations of mercury among bones were noted in rostrum (0.496 µg/g w.w.). In turn the smallest amount occurred in humerus (0.004 µg/g w.w.). Apart from rostrum and crista sterni all medians among bones were similar (Table 3). Differences only between rostrum and ulna and between rostrum and humerus were statistically significant (Kruskal Wallis test, *P*=0.0085). Significant correlations (Spearman R=0.9, *P*=0.0374) in mercury concentrations were found between femur and ulna, femur and radius, femur and tibia, and tibia and humerus.

Table 3 Mercury (µg/g w.w.) in bones and muscles of Mallard (n=5)

Bone	Minimum	Maximum	Median	Median	Maximum	Minimum	Muscle
ulna	0.008	0.053	0.016[a]	0.021	0.042	0.017	pectoralis major
radius	0.017	0.077	0.022[ab]	0.025	0.037	0.020	pectoralis minor
humerus	0.004	0.044	0.017[a]	0.021	0.033	0.016	gastrocnemius
femur	0.011	0.051	0.022[ab]	0.020	0.023	0.018	tibialis anterior
tibia	0.007	0.050	0.022[ab]	0.020	0.026	0.013	biceps
crista sterni	0.022	0.084	0.070[ab]	0.022	0.038	0.013	triceps
rostrum	0.054	0.496	0.213[b]	0.024	0.029	0.012	external oblique
				0.021	0.034	0.017	lingua

Legend: [a,b] different letters stress statistically significant differences

In the case of muscles all medians were similar – they fitted in the range between 0.021 and 0.025 µg/g w.w. (Table 3). Small differences among them were not statistically significant (Kruskal-Wallis test, *P*=0.9349). The highest concentration of mercury in muscles hit up to 0.042 µg/g w.w. (musculus pectoralis major). No statistically significant correlations were found in mercury concentrations among muscles.

Cadmium

Main place of cadmium deposit in the organism are kidney and liver. The most important factor which significantly influences its amount is an age **(White *et al*., 1978; Kalisińska *et al*., 2004)**. Parameters like sex and a type of shot (used for hunting) are recognized as not significant for cadmium concentrations **(Hiller and Barclay, 2011)**.

Cadmium concentrations in bones of Mallard from Zator in comparison to available world literature are not high. Observations led on three different water birds in South Africa showed that concentrations of this element in bone may hit even in 18.3 µg/g d.w. (*Fulica cristata* L.). According to our data, also concentration found in Reed Cormorant (range 4.3–5.8 µg/g d.w.) were higher **(Van Eeden and Schoonbee, 1996)**. However, **Kalisińska *et al*. (2004)** found in bones of Mallard from northern Poland visibly lower concentrations than these found by **Van Eeden and Schoonbee (1996)**. We cannot precisely compare them to our results because in many individuals of Mallard from Zator measured concentrations placed under the limit of detection of the used analytical method (what is for 0.5 g w.w. sample around 0.6 µg/g d.w.). It is worth to emphasize that a number of research of cadmium concentrations including bones are very limited, especially concerning different bones like this paper.

It is hard to assess the usefulness of bones as the food. This tissue is not edible but often the whole carcass is being cooked or roasted. In that case cadmium deposits may transfer to the dish. However, the only possibility of the evaluation the efficiency of this mechanism is to carry out an experiment similar to one described by **Mateo *et al*. (2007)**.

All medians and minimum values of cadmium concentrations in muscles are below the limit of detection of the used method (~0.6 µg/g d.w.). Similar concentrations for this species on the same area were noted in 2006 **(Binkowski, 2012a)**. Comparing our results to the literature we can assume that determined concentrations were comparable (below 0.6 µg/g d.w.) **(Gasparik *et al*., 2010)**. However, in comparison to concentrations found in Ancient Murrelet (sea bird of Alcidae family - *Synthliboramphus antiquus* Gmelin) where geometric mean run into 3.47 µg/g d.w., concentrations determined in Mallard from Zator were significantly lower **(Kim *et al*., 2009)**.

According to the European law **(EC, 2001)** the permissible concentration of cadmium in meat should not exceed 0.2 µg/g d.w. (originally 0.05 µg/g w.w.). Among all samples 7% have higher amount of cadmium per one gram of d.w. 93% of collected muscles accumulated cadmium in the level lower than the detection limit. In that case we cannot evaluate precisely their fitness for human consumption. Additional analyses with lower LoD of the method should be carried out.

Mercury

It is still unclear if mercury concentrations are influenced by the age and sex of individuals **(Świergosz, 1998; Hiller and Barclay, 2011)**. A connection with the type of shot used for hunting is not occurred **(Hiller and Barclay, 2011)**.

Mercury concentrations found by **Kalisińska *et al.* (2010)** in tarsus bone of goosander (*Mergus merganser* L.) fitted in the range 0.029–0.193 µg/g d.w. In Mallards from Zator medians of five bones were lower than minimum values noted by **Kalisińska**. However in the case of rostrum of Mallard median was higher than the maximum value measured in goosander.

Among muscles medians were very similar and low – all in the range 0.020–0.025 µg/g w.w. Maximum concentrations measured by **Gasparik *et al.* (2010)** were fitted around these values. Concentrations noted in southern Poland were lower than observed in Korea, where the maximum value was almost twice higher **(Lee *et al.*, 1989)**. Even higher concentrations found **Kalisińska *et al.* (2010)** in muscles of Goosander – the geometric mean run into 0.457 µg/g w.w. (originally 2.284 µg/g d.w.).

According to the safety norm **(EC, 2001)** which gives permissible concentrations of mercury in fishery products (officially only this kind of materials should be evaluated in the aspect of mercury) on the level of 0.5 µg/g w.w., all samples of Mallards from Zator were edible.

CONCLUSION

Among all samples of muscles 7% is not fit for human consumption because of cadmium concentrations. 93% of collected muscles accumulated cadmium in the level lower than the detection limit. These samples cannot be precisely evaluated as permissible food – another method with lower detection limit should be preceded. In the case of mercury all muscles were safe as food. Bones also did not accumulate high concentrations of this metal.

Acknowledgments: This work was done with the permission of the 1st Local Ethical Commission (for experiments on animals) in Cracow (decision number: 38/2012). Authors want to thank Tomasz Łaciak for his help in instrumental analysis. We are also very grateful to hunters from OHZ Zator for their voluntary help in the research.

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