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Effect of embedded shot on trace element concentrations in livers of Anseriformes species

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ABSTRACT

Trace elements were analyzed in the liver of white-fronted geese (*Anser albifrons*, n=15), mallards (*Anas platyrhynchos*, n=4) and spot-billed ducks (*Anas poecilorhyncha*, n=13) found dead in Gimpo, Korea. All mallards and eight spot-billed ducks had embedded lead shot. Embedded shot could be affected elevated trace element concentrations on geese and ducks. Element concentrations of cadmium (Cd), lead (Pb), chromium (Cr), aluminum (Al), copper (Cr), manganese (Mn) and zinc (Zn) differed among species and white-fronted geese without embedded shot had the lowest concentrations for all elements (geomean 0.36, 0.43, 0.07, 1.46, 7.60, 2.61 and 13.5 μg/g dw, respectively). Cadmium in four (3.27–7.77 μg/g dw) of 32 individuals and Pb in eight (5.07–9.72 μg/g dw) of 32 individuals exceeded a tentative threshold effect level of Cd (>3.0 μg/g dw) and Pb (>5.0 μg/g dw) for birds; all geese and ducks for Cr (0.07–0.43 μg/g dw) were within the background level (<4.0 μg/g dw). All trace element concentrations were much greater in waterfowl species with embedded shot than without shot. Essential trace elements such as Cr, Al (geomean 1.46–37.3 μg/g dw), Cu (7.60–57.1 μg/g dw), Mn (2.61–27.6 μg/g dw) and Zn (13.5–176 μg/g dw) were within the normal range and were probably maintained by normal homeostatic mechanisms.

1. Introduction

Waterfowl species are a large group of birds connected to wetlands and other water areas. These species can be threatened by numerous xenobiotics and elements which are often available from bottom sediments (Tylmann et al., 2007; Mountouris et al., 2002). Sediments play a role as carriers and deposits of various trace elements (Singh et al., 2005). Because waterfowl species may ingest sediment when they are foraging, they may be exposed to non-essential elements that are deposited in and associated with sediments in wetlands (Taggart et al., 2005). Also, essential and non-essential elements in waterfowl species can be accumulated through ingestion of lead shotgun pellets (Ferreyra et al., 2015), lead sinkers (Scheuhammer and Norris, 1996) and by food-chain transfer (Kim and Oh, 2012). Therefore, waterfowl species have been recommended as useful bio-indicators for monitoring of trace elements (Sinka-Karimi et al., 2015; Ferreyra et al., 2015). In trace elements, the bioaccumulation of non-essential elements including Cd, Pb and Cr may be associated with neurological and physical problems including smaller clutch sizes, reduced fertility, hatching failure and nestling mortality (Binkowski et al., 2013; Janssens et al., 2001).

In this study, we examined 32 individuals of white-fronted geese (*Anser albifrons*), mallards (*Anas platyrhynchos*) and spot-billed ducks (*Anas poecilorhyncha*) found dead in paddy fields near Gimpo, Korea in 2012. Gimpo (Han River estuary) is well known as an important wintering habitat for various waterfowl, gull species and white-naped cranes (*Grus vipio*). Also, the wintering birds in Gimpo are exposed to legal and illegal hunting, Pb contamination from embedded Pb shot and ingestion of Pb pellet. Ingestion of Pb sinkers is another important pathway to Pb exposure and poisoning (Kim and Oh, 2012). In Korean studies, white-fronted geese (geomean 5.26 μg/g dw) exceeded the threshold level of abnormal exposure (>5 μg/g dw) in livers for waterfowl (Guitart et al., 1994), but eurasian wigeons (*Anas penelope*), mallards and spot-billed ducks did not exceed (geomean 3.72, 3.82 and 4.53 μg/g dw, respectively) (Kim and Oh, 2012).

Birds may differ in accumulation and excretion of trace elements and this may be associated with different diets and trophic levels, or physiological and ecological species-specific element requirements (Hofer et al., 2010). Also, geese and ducks had different element concentrations among the species in Korea (Kim and Oh, 2012). We hypothesized that geese and ducks with

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embedded shot have elevated trace element concentrations. The objective of this study was to assess the accumulation of seven trace elements (Cd, Pb, Cr, Cu, Al, Mn and Zn) in liver tissues of three waterfowl species with or without embedded shot and to determine whether non-essential element (Cd, Pb and Cr) concentrations exceed estimated toxicity thresholds.

2. Materials and methods

2.1. Study site and sampling

We collected 15 carcasses of white-fronted geese, 4 mallards and 13 spot-billed ducks from Gimpo (36°63′N, 126°72′E), Gyeonggi-do, Korea in February 2012. In these specimens, we found a Pb shot in 8 spot-billed ducks and 4 mallards embedded in the flesh. The other causes of death were unknown. White-fronted geese included 11 adults and 4 juveniles, spot-billed ducks included 10 adults and 3 juveniles and all mallards were adults.

The waterfowl were weighed (± 0.1 g), stored in chemically clean plastic bags, and frozen (-20 °C) until they were dissected and analyzed. The liver was removed from the carcass, weighed (± 0.1 g) and dried in an oven for 24 h at 105 °C until no further weight reduction occurred. Liver remainders were homogenized in a glass Teflon homogenizer and weighed (± 0.1 g). All trace element concentrations (μg/g) in the liver were estimated on dry weight (dw) basis.

2.2. Trace element analysis

Approximately 2–3 g of each sample was digested in the presence of a mixture of concentrated nitric, perchloric and/or sulfuric acids in Kjeldahl flasks. Aluminum, Cu, Mn, Zn and Fe concentrations were determined by flame atomic absorption (AA) spectrophotometry (Hitachi Z-6100), after mineralization. Cadmium, Pb and Cr concentrations in the digested solutions were extracted with DDTC (Sodium N, N-Diethyldithio-carbamate trihydrate ($(C_2H_5)_2NCS_2Na \cdot 3H_2O$)-MIBK (Methyl Isobutyl Ketone ($CH_3COCH_2CH(CH_3)_2$) and determined by flame atomic absorption (AA) spectrophotometry (Hitachi Z-6100) (Kim and Koo, 2007).

Eight spikes and blanks were included in the analysis (about 20% of the total number of samples). A spike, a blank, a standard (0.01, 0.02, 0.03, 0.04, 0.05 ppm for Cd and Cr; 0.1, 0.2, 0.3, 0.4, 0.5 ppm for Pb and 1, 2, 3, 4, 5 ppm for Al, Cu, Mn and Zn) and a sample were run in triplicate in each analytical run. Spikes recoveries ranged from 94% to 106%. Recovered concentrations of the samples were within 5% of the standard error. Detection limits were 0.01 μg/g for Cr and Cd, 0.1 μg/g for Pb and 1.0 μg/g dry weight (dw) for Al, Cu, Mn and Zn.

2.3. Statistical analysis

We statistically tested trace elements for differences among waterfowl species using one-way analysis of variance (ANOVA). The Bonferroni mean separation tests was used to separate species means following a significant ANOVA. Differences in trace element concentrations between waterfowl species with embedded shot and without shot were tested using *t*-tests. Data were log transformed to obtain a normal distribution that satisfied the homogeneity of variance assumptions of ANOVA and *t*-test (Kim and Koo, 2007; Kim and Oh, 2012). We present geometric means, 95% confidence intervals, arithmetic mean and standard deviation in tables and texts. Statistical analyses were carried out using SPSS 13.0 version.

3. Results

3.1. Concentrations of trace element

Cadmium and Pb, Cr, Al, Cu, Mn and Zn concentrations were significantly different among waterfowl species (ANOVA, $p < 0.001$, respectively) (Table 1). First, Cd and Mn concentrations in spot-billed ducks without (geomean 1.57 and 27.6 μg/g dw, respectively) and with shot (1.60 and 20.9 μg/g dw) were higher than measured in mallards with shot (0.85 and 16.4 μg/g dw) which in turn were higher than in white-fronted geese without shot (0.36 and 2.61 μg/g dw). Second, for Pb and Zn, mallards with shot (geomean 4.27 μg/g dw and 176 μg/g dw, respectively) and spot-billed ducks with (4.44 and 157 μg/g dw) and without shot (3.44 and 157 μg/g dw) had greater Pb and Zn concentrations than in white-fronted geese without shot (0.43 and 13.5 μg/g dw). Third, Cr and Al concentrations were higher in spot-billed ducks without shot (geomean 0.43 and 37.3 μg/g dw, respectively) than measured in spot-billed ducks (0.23 and 14.8 μg/g dw) and mallards (0.17 and 12.5 μg/g dw) with shot which turn were higher

Table 1
Trace element concentrations (geometric mean, 95% confidence intervals (CIs) and mean ± SD, μg/g dw) in livers of waterfowl species, Korea.

	Cd	Pb	Cr	Al	Cu	Mn	Zn
White-fronted geese (n=15) (no shot)							
Geomean	0.36C[1]	0.43B	0.07C	1.46C	7.60C	2.61C	13.5B
CIs	0.31–0.42	0.20–0.67	0.06–0.08	0.54–2.38	0.57–43.2	0.46–6.34	1.51–32.5
Mean ± SD	0.72 ± 1.11	3.32 ± 2.61	0.11 ± 0.13	5.44 ± 11.1	111 ± 362	18.9 ± 45.9	91.5 ± 215
Mallards (n=4) (Embedded shot)							
Geomean	0.85B	4.27A	0.17B	12.5B	34.9B	16.4B	176A
CIs	0.37–2.38	0.22–8.32	0.17–0.18	4.05–21.0	23.2–46.6	5.82–27.1	158–193
Mean ± SD	1.15 ± 1.10	4.74 ± 2.92	0.17 ± 0.01	13.3 ± 6.12	35.4 ± 8.44	17.3 ± 7.66	176 ± 12.6
Spot-billed ducks (n=5) (No shot)							
Geomean	1.57A	3.44A	0.43A	37.3A	27.9B	27.6A	157A
CIs	0.85–2.29	3.17–3.71	0.29–0.58	19.9–86.4	25.5–30.3	11.5–43.7	129–185
Mean ± SD	1.61 ± 0.52	3.45 ± 0.20	0.44 ± 0.10	44.9 ± 35.4	27.9 ± 1.73	28.8 ± 11.6	158 ± 20.1
Spot-billed ducks (n=8) (Embedded shot)							
Geomean	1.60A	4.44A	0.23B	14.8B	57.1A	20.9A	157A
CIs	0.24–2.96	1.88–7.00	0.10–0.37	4.59–29.9	12.6–102	13.1–28.8	71.6–243
Mean ± SD	2.03 ± 1.55	5.07 ± 2.92	0.26 ± 0.15	19.7 ± 17.3	68.4 ± 50.8	22.5 ± 8.94	177 ± 98
P value[2]	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1] Means sharing same letter were not significantly different among species and condition.
[2] *P* value for ANOVA comparing among species.