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Comparing erosion and organ accumulation rates of lead and alternative lead-free ammunition fed to captive domestic ducks

Oliver Krone, Norbert Kenntner, Nicole Ebner, Claudia A. Szentiks, Sven Dänicke

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Abstract Lead poisoning of birds of prey from ingestion of ammunition lead has been well documented. Alternative, lead-free ammunition is widely available, but the toxicokinetics of other metals in birds are poorly understood. We tested the erosion of lead, copper, zinc, iron and brass by feeding domestic Pekin ducks (*Anas platyrhynchos* forma *domestica*) defined numbers of small metal pellets. The accumulation of these metals was analysed in the breast muscle, brain, pancreas, liver and kidney. Four weeks after application, the ducks were euthanized and necropsied, internal organs tested for metal accumulation and gizzard pellets collected and weighed to record loss by erosion. Degree of erosion was highest in zinc pellets (81% mass loss), followed by iron (46%) and lead (45%) and was only marginal in copper and brass. Only lead showed highly elevated levels of accumulation in organs compared to controls.

Keywords Brass · Bullet · Copper · Hunting ammunition · Iron · Lead · Lead poisoning · Pellet · Zinc

INTRODUCTION

Lead poisoning of birds of prey from hunting ammunition is a well-documented and worldwide problem (Fisher et al. 2006; Mateo 2009; Pain et al. 2009; Krone 2018). Several studies have demonstrated the toxicity of lead in birds (Pain 1992, 1996; Ringelman et al. 1993; Scheuhammer and Norris 1995; Kelly et al. 1998; Levengood et al. 1999; Mitchell et al. 2001; Brewer et al. 2003). Alternative lead-free bullets often consist of copper and its alloys, whereas shot gun pellets often consist of zinc and iron. For those alternatives, little information exists on the erosion, kinetics and toxicology in birds. Other alternatives for shot gun pellets such as bismuth and tungsten have already been approved USFWS (2006). Conventional bullets contain a lead (Pb) core that fragments on its way through the shot animal resulting in a particle cloud. Highest lead contamination is found around the wound channel, but fragments can reach all tissues. Viscera taken out of shot animals and dead but not retrieved game animals constitute the most important source for lead contamination in birds of prey and scavengers (Trinogga et al. 2013). Shot and wounded small game easily become prey for raptors and might carry pellets in their body. Active hunting raptors as well as scavengers ingest the small bullet particles and the pellets of shot guns with their meal and fall victim to lead poisoning.

Here, we study the erosion of the metals used as ammunition such as lead, but also alternative metals (copper, brass, zinc and iron) in ducks as surrogates for birds of prey. Since we know that lead accumulates in liver and kidney, we want to understand to what extent alternative metals accumulate in certain organs (liver, kidney, breast muscle, brain, pancreas).

MATERIALS AND METHODS

During the 4-week trial, we used six groups, each of 40 Pekin ducks (*Anas platyrhynchos forma domestica*), including one control group, totalling 240 birds. Ducks were kept in cages with ground litter, food and water for ad libitum consumption. This meant that feeders always contained feed which consisted of commercial duck feed. The experiment started after a 3-week familiarization phase. The groups were separated depending on the type of metal pellets ($\varnothing \pm 3.3$ mm) administered: lead (Pb), copper (Cu), zinc (Zn), iron (Fe) and brass ($CuZn_x$).

Table 1 Sizes and weights of each test shot (n = 20 per shot) used for avian toxicity tests

Group	1	2	3	4	5	6
Metal	Control	Copper	Brass	Zinc	Lead	Iron
No. pellets/duck	0	6	6	6	6	6
Size ± SD [mm]	–	3.15 ± 0.05	3.0 ± 0.00	3.05 ± 0.14	3.2 ± 0.05	3.2 ± 0.00
Weight ± SD [mg]	–	148 ± 0.33	120 ± 3.2	105 ± 12.3	191 ± 7.1	138.6 ± 1.0

Table 2 Metal composition of tested gun shot pellets used in the duck experiment, as analysed by an XRF-spectrometer. Means are based on analyses from three pellets of each metal type

	Zn	Cu	Fe	Mn	Sn	Pb	Sb	As
Brass shot	32.48	67.52						
Iron shot			99.62	0.37				
Zinc shot	98.06				1.94			
Lead shot						95.85	2.99	1.09
Copper shot		100.00						

Conventional hunting ammunition was purchased from Jagd-und Sportmunitions GmbH (Germany) for zinc and lead pellets from Rottweil Tiger (Germany) in the sizes recommended to test non-toxic pellets by Environment Canada (1993), similar to the US #4 shot size (~ 3.3 mm). Cartridges were opened and the metallic pellets used for the experiment were removed. Six shotgun pellets were placed in the gizzard of each duck of the experimental groups by oral intubation (Table 1). The general behaviour of ducks and their feeding behaviour were observed on a daily basis. At weekly intervals, the birds were weighed. After 1 and 7 days, the retention of pellets was controlled by radiographing the ducks. In ducks with fewer than 6, missing pellets were replaced until the initial number was reached again. At the end of the 4-week experiment, the ducks were euthanized, necropsied and internal organs prepared for histological examination. Gizzards and intestines of all birds were washed and sieved to screen for retained shot pellets. During routine necropsy, body condition and sex of each duck were also determined. Retained pellets were measured and weighed. Organ samples for histo-pathological examinations were immediately fixed in buffered formalin, whereas those for metal analysis were kept at − 20 °C prior to microwave-assisted acid digestion. We utilized graphite furnace Atomic Absorption Spectroscopy (AAS, Analytik Jena ZEEnit 700) for measuring lead concentrations, and flame AAS methods for analysing copper, zinc and iron in the liver, kidney and brain of the Pekin ducks. Statistical analyses were performed in Systat 11 (Systat Software Inc., CA, USA). A Tukey–Kramer multiple comparison test was used to compare values between the different organs in the metal exposure groups as well as between the organs of the treated (n = 40 for each metal) and control group (n = 40). Organ metal concentrations are expressed as wet weight (ww).

The different pellets were analysed in a micro X-ray fluorescence (XRF) spectrometer for zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), tin (Sn), lead (Pb), antimony (Sb), nickel (Ni) and bismuth (Bi) described by Wolff (2009). The XRF analysis is based on special optics for concentrating X-rays in small sample areas of µm-sized spots and uses Silicon-Drift-Detectors which can handle very high count rates (Haschke et al. 1998). To analyse the composition of the shot gun pellets used in our experiment, three pellets of each type have been analysed by a table-operating micro-XRF spectrometer from Bruker, Berlin, Germany. Detection limit was set at 5 ppm (Table 2).

RESULTS

The metal shot gun pellets administered in the gizzard of the Pekin ducks showed significant differences in the mass retained after the 4-week experiment. The brass and copper pellets lost only 1.4 and 1.5% of their original mass, whereas the lead, iron and zinc pellets lost 45.3, 45.8 and 81.4%, respectively (Figs. 1, 2). The lead and zinc pellets lost on average 3.1 mg of their mass per day, iron 2.3 mg and copper and brass less than 0.1 mg.

Internal organs (breast muscle, pancreas, brain, kidney, liver) showed elevated levels of lead in the lead-exposed group. The remaining four groups exposed to copper, zinc, brass and iron showed no accumulation for other metals (Fig. 3a–e).

In the lead burdened group, lead contents were found to be significantly higher in liver, kidney and pancreas

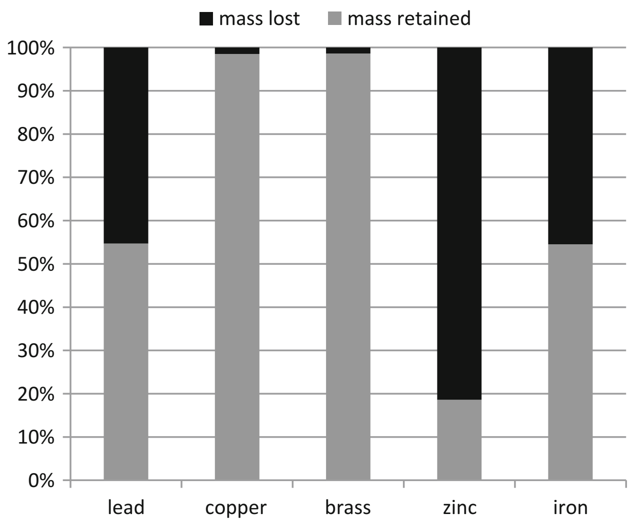

Fig. 1 Remaining mass of metallic shots (lead, copper, brass, zinc and iron) after 4-week exposure in the gizzards of Pekin ducks

compared to breast muscle and brain (Tukey–Kramer multiple comparison test, $p < 0.001$). When comparing the values in the corresponding organs between the lead and the control group, the values differed significantly between the livers, kidneys and pancreas, but not between the breast muscles and the brains ($p < 0.001$). In the group where copper was administered, the liver showed significant higher concentrations of copper in comparison to kidney ($p < 0.001$), pancreas ($p < 0.001$), breast muscle ($p < 0.001$) and brain ($p < 0.001$), but not between the other organs. Comparing the copper values between the exposure and control group no significant differences could be detected in the corresponding organs. Zinc was accumulated in the pancreas to a significantly higher degree than in liver (p < 0.05), kidney ($p < 0.001$), breast muscle ($p < 0.001$) and brain ($p < 0.001$) in the ducks fed with zinc. The comparison of the corresponding organs between the zinc and control group only revealed differences for the pancreas ($p < 0.001$) but not for the remaining organs. In the iron-loaded group, iron values were higher in the liver than in the kidney ($p < 0.001$), pancreas ($p < 0.001$), breast muscle ($p < 0.001$) and brain ($p < 0.001$). Kidney values were higher than in the pancreas ($p < 0.01$), breast muscle ($p < 0.01$) and brain ($p < 0.001$). No differences in iron values were detected between the corresponding organs in the iron challenged and the control group.

No significant loss of body weight was detected in the individual ducks groups challenged with pellets in the gizzard of any metal or in the control group. Histo-pathological examinations of the organs (breast muscle, lung, heart, gizzard, intestine, spleen, pancreas, brain, kidney and liver) revealed no group-specific or consistent alterations. All birds (n = 240) survived and were found in a very good nutritional status at the end of the experiment.

For differences between organs, we measured the lead concentrations in liver, kidney, pancreas, breast muscle and brain of the group exposed to lead for 4 weeks. Highest mean (median) values were detected in the kidney, liver and pancreas with 12 127 (10 159) ppb, 11 253 (9518) ppb and 10 750 (8033) ppb. The lowest lead concentrations were found in the breast muscle and brain, with 322 (295) ppb and 788 (639) ppb (Fig. 4).

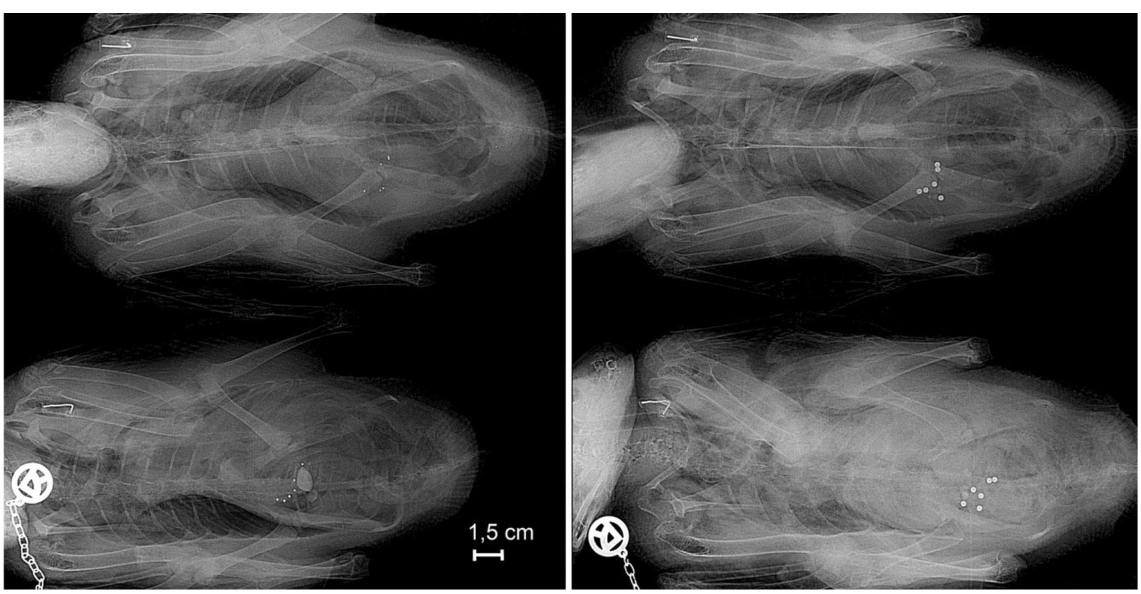

Fig. 2 Radiographs of four Pekin ducks following 4-week exposure to six zinc pellets (left side) and six copper pellets (right side). Reduction in size and mass is visible in case of zinc pellets only

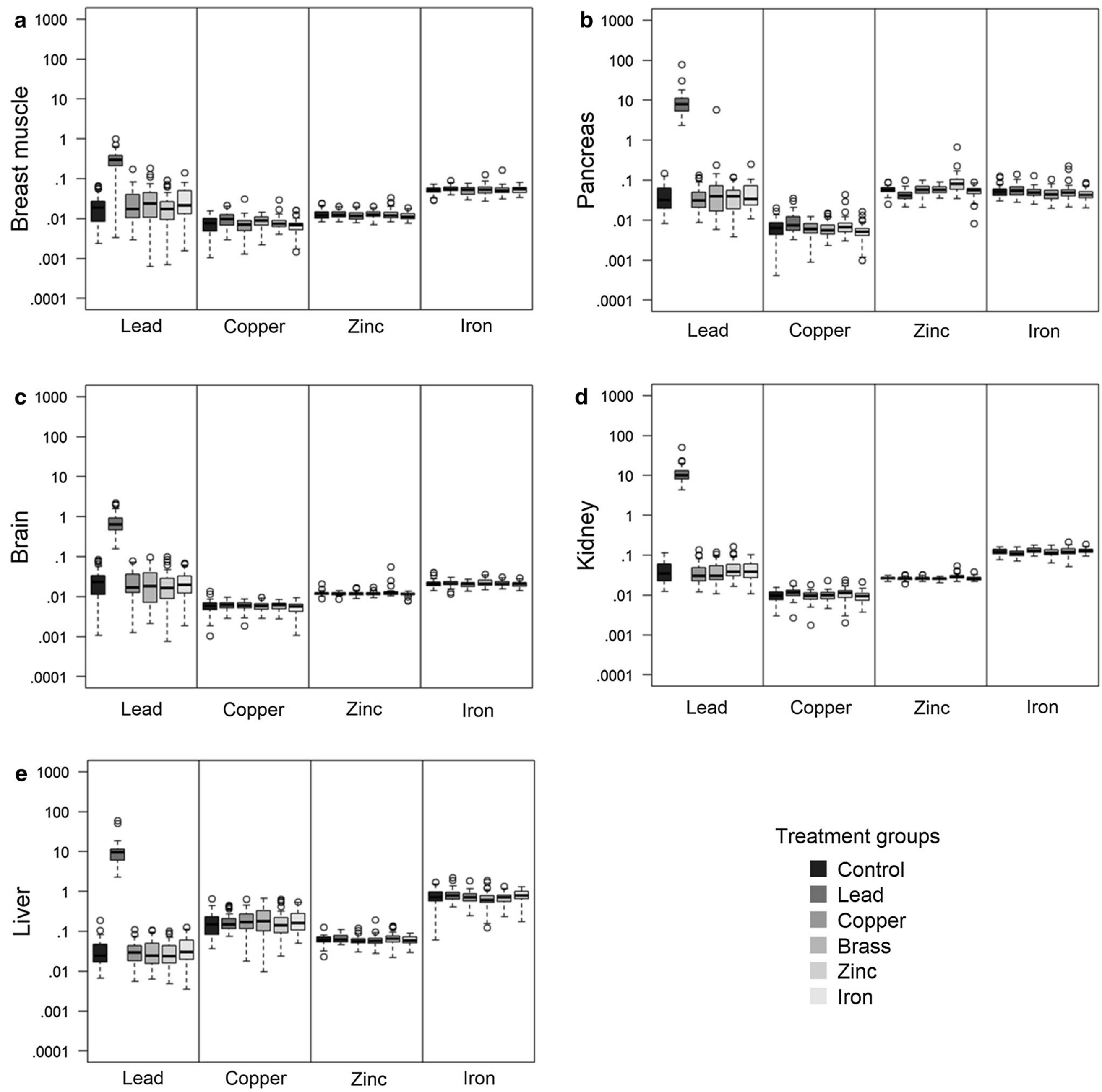

Fig. 3 **a–e** Organ concentrations of lead, copper, zinc and iron measured for each of the six experimental groups. Each group consisted of 40 Pekin ducks each fed six shots of lead, copper, brass, zinc and iron. The first group is the control group. All values are given in ppm

DISCUSSION

Our results clearly demonstrate that three of the five metals/alloys were eroded in the gizzards of Pekin ducks during the 4-week experiment. Lead, iron and zinc lost 45 to 81% of their mass, whereas copper and brass around 1.5% only. The highest losses were associated with zinc, lead and iron pellets, whereas copper and brass pellets retained most of their mass. There were no increases in detected levels of the above-mentioned elements in selected tissues (breast muscle, pancreas, brain, liver, kidney) for any element with the exception for lead. There were significant increases of lead in liver, kidney and brain tissue. Breast muscle and brain accumulated lead to a much smaller degree but these values did not differ significantly between the lead exposure and control group. Death, obvious clinical signs or alteration of a normal behaviour were not observed in any of the Pekin ducks during the 4-week trial. At the end of the experiment, all ducks were in good shape and had good nutritional status, indicating

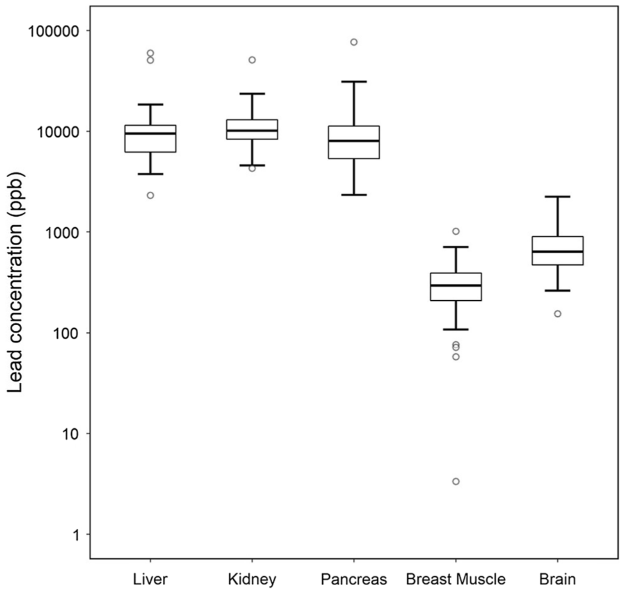

Fig. 4 Lead (Pb) concentration in liver, kidney, pancreas, breast muscle and brain of Pekin ducks exposed to lead for 4 weeks. Values are given in ppb

that no metal/alloy affected the health of the birds in any measurable way.

Zinc had the highest mass loss in the gizzard among all pellets tested, but only the pancreas showed a significant accumulation in the treated group compared to the control group. Mean zinc levels in the organs of ducks from the zinc group were 69.68 µg/g, 29.56 µg/g and 101.62 µg/g for liver, kidney and pancreas, which were within the expected physiological range, and below the toxic levels of about 400 (360–402) µg/g, 300 (268–360) µg/g and 2000 (2071–2255) µg/g for liver, kidney and pancreas, respectively, as reported by Levengood et al. (1999). In contrast to our study, Levengood et al. (1999) reported a mortality of 45% in male and 80% in female mallards (*Anas platyrhynchos*) fed with six zinc pellets each. In those ducks that survived there was a mass decline of 88.1%, which is slightly higher than in our study. However, French et al. (1987) had similar results compared to our study when they fed six to ten zinc pellets to mallards. They did not report any mortality, signs of disease or organ alterations. However, Sileo et al. (2004) reported lethal zinc values in the pancreas of wild waterfowl ranging from 260 to 2400 mg/kg (dw) for three Canada geese and 440 mg/kg (dw) for a mallard resulting in a pancreatitis. From our experiment, we conclude that zinc is only dissolved in the bird's gizzard in its pure form and not from brass (copper–zinc alloy) in which form it is not bioavailable. The zinc pellets we used in our experiment contained 1.94% tin. Because of its known toxicity, zinc should not be considered as alternative for hunting ammunition in its elementary form, but might be useful as alloy (e.g. copper–zinc).

Lead pellets also had a high propensity to erode in the gizzard of Pekin ducks and showed 45.3% mass loss during the 4-week experiment. In contrast to other soluble elements, we found a significant organ accumulation of lead, which was most distinctive in the liver, kidney and pancreas and to a much lesser extent also in the breast muscle and brain. With concentrations exceeding 6 ppm (ww) in liver and kidney, threshold levels indicative of clinical poisoning in free-living water birds (Pain 1996) had been reached in our domestic Pekin ducks. Liver concentrations of more than 10 ppm might be already indicative for severe clinical poisoning in Anseriformes (Franson and Pain 2011). Finley and Dieter (1978) found a dose-related mortality in mallards given commercial lead pellets. Depending on the size and number of pellets administered they reported 5% to 50% mortality. However, domestic ducks are more tolerant of lead than wild ducks (Jordan and Bellrose 1950). In order to demonstrate lethal effects and severe loss of weight in the ducks, Jordan and Bellrose (1950) had to significantly increase the number of lead pellets from 5 to 25 given to the Pekin ducks in their experiment. Our ducks were fed to maintain them in a good nutritional state and the commercial feed used contains high levels of calcium, known to moderate the effects of lead. Both factors may have contributed to their better physiological status compared to other such studies (Meredith et al. 1977; Scheuhammer 1987). The important relationship between diet and lead poisoning has been reported several times (Clemens et al. 1975; Jordan and Bellrose 1950). The lead pellets used in our experiment contained 2.99% antimony and 1.09% arsenic, but we think this had no effect on the ducks which is in concordance with the results reported by Jordan and Bellrose (1950).

Iron pellets in the gizzards of ducks were reduced by 45.8% in their mass, but iron levels were not elevated in any of the organs measured. Concentrations are considered within the expected physiological range and were similar in all groups tested. Probably most iron was excreted with the faeces before being absorbed, which is the case under normal conditions (Hooser 2012). Clemens et al. (1975) reported an erosion of only 9% in their steel pellets administered to mallards at the end of a 3-week experiment. The iron pellets used in our study contained 0.37% manganese, which we believe had no apparent effect on the health of our Pekin ducks.

Copper and brass pellets lost only 1.5% mass by erosion. No accumulation of copper could be detected in any organs measured. Values were considered to fall within the limits of concentrations expected under normal physiological conditions. In domesticated mallards fed diets supplemented with high copper loadings, a dose- and time-dependent increase in copper concentrations has been found with a maximum concentration of 254 mg Cu/kg dry weight (dw) in the livers (Eisler 1998). Our copper pellets were pure (100%) and we could not detect any other metal in the sample using the XRF-spectrometer.

Interpretation and applicability

The physiology of the digestive system differs between herbivorous and carnivorous birds. The major differences are a very muscular gizzard containing grit and small stones to mechanically break up plant fibres in most herbivorous birds including ducks and a less muscular but more acid gizzard in birds of prey. Acid fluids in the duck's gizzard have a pH value of around 2.5 and raptors have a pH value of $\sim$ 1.8 (Locke and Thomas 1996). Despite the fact that the mechanisms of breaking down and dissolving metallic particles including lead are not the same, absorption of metallic elements in general is assumed to be almost identical. Both groups, i.e. water birds (including ducks and swans) and birds of prey suffer from lead intoxication caused by the ingestion of metallic lead bullet fragments and pellets from hunting ammunition. Therefore, we interpret the data reported above in the results section to be valid for both groups which means that the ducks used in our experiment can be considered as surrogates for birds of prey in the strict sense of our trail. The alternative metals and alloys tested here strongly varied in their retention mass. Copper and brass only lost 1.5% of their originally mass, which makes them useful alternative ammunition. The iron pellets lost 45.8%, but iron orally ingested can be considered harmless to the birds, making iron also a good alternative. Zinc lost 81.4% of the originally mass and no adverse effects on the ducks could be found, but zinc as an alternative ammunition material should be considered with extreme care. The detrimental effect of lead on birds has been repeatedly reported and a mass loss of 45.3% in the ducks' gizzard shows the potential to dissolve critical amounts.

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Research involving animal rights The trail of studying the erosion, accumulation and toxicity of the different metals and alloys used as material for hunting ammunition in captive ducks under controlled conditions has been approved by the responsible state agency: Lower-Saxony State Office for Consumer Protection and Food Safety under the registration number 33.11.42502-04-084/07.

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AUTHOR BIOGRAPHIES

Oliver Krone (✉) is a wildlife veterinarian and raptor ecologist working at the Leibniz-Institute for Zoo and Wildlife Research. His interest is in conservation medicine with a special focus on top predators. He studies infectious diseases as well as toxicological aspects. He aims to enhance nature conservation by mitigating human–wildlife conflicts.
Address: Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research (IZW), P.O. 601103, 10252 Berlin, Germany.
e-mail: krone@izw-berlin.de

Norbert Kenntner worked as a wildlife toxicologist studying chlorinated hydrocarbons and heavy metals in birds of prey at the Research Institute of Wildflife Ecolgy in Vienna and the Leibniz-Institute for Zoo and Wildlife Research, Berlin. Actually he is risk manager at the Federal Office of Consumer Protection and Food Safety. In his leisure time he leads a longterm color-ringing project on urban goshawks in Berlin.
Address: Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research (IZW), P.O. 601103, 10252 Berlin, Germany.

Nicole Ebner studied biology and holds MSc from Alexander von Humboldt University Berlin and a PhD from Charité Medical School. During her diploma thesis she worked at the Leibniz-Institute for Zoo and Wildlife Research. Since 2014 she works as a Research fellow at the University Medical Center Göttingen, Department of Cardiology.
Address: Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research (IZW), P.O. 601103, 10252 Berlin, Germany.

Claudia A. Szentiks is a veterinary pathologist working at the Leibniz-Institute for Zoo and Wildlife Research. In addition to necropsies on zoo and wild animals, she is running a long-term monitoring project on diseases and causes of death in free-living wolves in Germany.
Address: Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research (IZW), P.O. 601103, 10252 Berlin, Germany.

Sven Dänicke is an animal nutritionist at the Institute of Animal Nutrition Braunschweig, which belongs to the Federal Research Institute for Animal Health (Friedrich-Löffler-Institut). His main interest is to investigate the associations between nutrition and health of animals. Amongst others, he is experienced in conducting feeding experiments with farm animals in general and with poultry in particular aimed at investigating the effects of unwanted compounds in feed on health and food safety.
Address: Institute of Animal Nutrition (ITE), Bundesallee 37, 38116 Braunschweig, Germany.