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Pilot study on metal contents in meat portions from wild game killed by “lead-free” rifle bullets

Pilotstudie zu Metallgehalten in Fleischportionen von mit „bleifreien“ Büchsengeschossen erlegtem Wild

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Summary

108 meat packs (obtained from hunted animals and from pork shoulder experimentally contaminated by shooting on it with bullets) were examined for radiodense fragments and after storage and boiling, subjected to simulated gastric and duodenal digestion. In both the digested and undigested fraction, contents of Al, Cr, Cu, Fe, Ni, Pb, Zn were determined. Whereas the metal contents in the digested fractions did not differ markedly from those in controls, higher metal contents were detected in undigested residues of three samples. But even in the meat portions with high metal contents, the bioavailable fraction of metals was in the same range as in meat portions with low metal levels. Although the data originate from a small sample set and should be considered a preliminary finding, the approach of studying meat portions instead of small aliquots – and the assessment, which fraction of metal particles will actually be available for absorption – has its merits, when metal contamination is inhomogeneous.

Keywords: Meat from wild game, metal from rifle bullets, alimentary exposure

Zusammenfassung

108 Fleischportionen von erjagtem Wild und aus experimentell beschossenen Schweinsschultern wurden auf röntgendichte Partikel untersucht und, nach Lagerung und Kochen, einer Verdauung mit künstlichem Magen- sowie Dünndarmsaft unterzogen. Während die Metallgehalte in den verdauten Fraktionen sich nicht merklich von jenen der Kontrollgruppe unterschieden, wurden höhere Metallgehalte in den unverdauten Rückständen von drei Proben gefunden. Aber selbst in diesen Fleischportionen mit den hohen Metallgehalten lag die verfügbare, d. h. in der flüssigen Phase vorliegende Metallmenge, auf niedrigem Niveau. Obwohl die Daten von einer kleinen Probezahl ausgehen und als vorläufig betrachtet werden sollten, hat die Vorgangsweise der Untersuchung ganzer Fleischportionen statt aliquoter Anteile eines Homogenisates und die Differenzierung, welche Anteile der Metallpartikel nun wirklich zur Resorption verfügbar sind, ihre Vorzüge, insbesondere im Falle inhomogener Metallverunreinigungen.

Schlüsselwörter: Wildfleisch, Geschoßmetall, alimentäre Exposition

Introduction

It has been established that the use of lead-containing rifle bullets for hunting large game causes lead deposits in edible tissues, either in form of "lead dust" or as larger fragments (Hecht, 1984; Dobrowolska and Melosik, 2008). Disposal of lead-containing offal or trimmings of game will have detrimental effects on other wildlife species, in particular birds of prey (Fisher et al., 2006), which feed on such offal. In addition, elevated lead levels in meat from wild game can be considered a health hazard for human consumers (Hunt et al., 2009; Iqbal et al., 2009; Knott et al., 2010), although the actual assessment of the hazard is depending on the amount and frequency of meat consumed in relation to other sources of alimentary lead (BfR, 2009), mode of culinary processing (Mateo et al., 2007, 2011) and the susceptibility of the consumer groups (BfR, 2010). These findings have renewed interest in the development and use of "lead free" rifle bullets and in the construction of bullets, which will deposit energy in the target tissue not by fragmenting, but rather by deformation while retaining (most of) their mass. Although such issues have been studied in the 1980ies (Hecht, 1984), it was the obvious inadequate killing power of "lead free" deforming bullets at these times which impeded further research in Europe. Not much is known about the release of metals from non-lead fragments embedded in meat, and what amount of metals would be available, when such meat is processed and ingested by humans.

The aim of this pilot study was, therefore, to determine metal contents of meat portions originating from hunted wild game and from pork tissues with experimental metal contamination and to compare these amounts with those in non-contaminated meat portions. In particular, the meat portions were subjected to simulated gastric and duodenal digestion, and the metal amount in the liquid phase was assumed to represent the bioavailable metal fraction, i. e. the actual consumer exposure. The metal contents in solid residues would represent the fraction being excreted via feces.

Materials and Methods

Shoulder muscle (pork) subjected to bullet metal contamination under standardized conditions

Three pork shoulders (from landrace crossbreeds of 90–95 kg carcass weight, 24 h post mortem) were obtained from a local slaughterhouse. Each shoulder was suspended from a rack, and the location of the shoulder joint was marked with a pen. From a 40 m distance, 2 bullets of the same type [Exergy (XRG), Sellier & Bellot, Vlasim, Czech Republic; Bionic Yellow (BY), RUAG Ammotech, Fürth, Germany; all 0.308 inch Winchester calibre) were fired on each shoulder aiming at the marked region. It was decided to fire 2 shots per shoulder in order to have a higher probability for hitting bones and producing metal fragments.

Shoulders were then examined via X-ray to study if dense fragments (indicating metal or bone fragments) were present within the tissue (Irschik et al., 2013). Further processing of shoulders was done by a butcher, simulating commercial practice, i. e. trimming of the shot wound, removing all lacerated tissue and skinning of the shoulder. Meat was cut to 90 g portions, which were vacuum-packed and stored for 7 days at 0–2 °C. Portions were examined via X-ray and all portions with radiodense particles plus one "blank" portion per shoulder (see Tab. 1) were subjected to boiling (in vacuum package, 72 °C internal temperature, cooling to room temperature) and then subjected to artificial digestion.

Metal contents in shoulder muscle portions from hunted roe deer and wild boar

Meat portions of 90 g weight were obtained from the shoulder regions of 6 roe deer and 2 wild boars, which had been hunted with XLC (bullet by Barnes Bullets, Mona, USA, loaded by Sellier & Bellot, Vlasim, Czech Republic) and XRG bullets of 0.308 inch diameter (Tab. 1). Shooting distance had varied from 25 to 40 m, and all shots had passed the thorax and had damaged the bones of at least one shoulder. After evisceration, bruised tissue around the shot wound was removed, and the remaining shoulder and anterior chest meat was portioned in 90 g units, which were vacuum-packed and stored at 0–2 °C for 7 days post mortem.

The number of meat packages containing radiodense particles (detected according to Irschik et al., 2013) is reported in Table 1. All packages were boiled in water to reach 72 °C internal temperature and then cooled down.

Simulated gastric and duodenal digestion

Meat portions were minced and digested exactly according to Mateo et al. (2011), with upscaled quantities of reagents. Enzymes were from Sigma-Aldrich, whereas other reagents were from Roth, Germany. All meat packs with dense particles were digested and examined for metal contents, and per animal, 1 meat pack without dense fragments was included as control.

In brief, 90 g meat plus 270 ml gastric juice (pH 3 ± 0.1) were incubated under shaking for 2.5 h at 37 °C; then, 9 ml duodenal juice were added, pH was adjusted to 6.5 ± 0.1 and digestion continued for 2 h at 37 °C. During digestion, the headspace in the vessel was purged with nitrogen. The slurry was then divided in two parts, which were centrifuged at 2000 g for 10 min at ambient temperature (Sorvall SH-3000; Sorvall, USA). The metal contents in the digestive fluid and the undigested solid residue were determined.

TABLE 1: *Details on meat samples.*

Species	Bullet, shooting distance, carcass weight (with head)	# of 90 g meat portions produced from shoulder, rib and caudal neck	# of portions which contained radiodense fragments	# of portions with high metal content
Roe deer, #				
1	XLC, 35 m, 12 kg	11	4	0
2	XLC, 40 m, 15 kg	4	2	0
3	XRG, 35 m, 11 kg	13	9	1
4	XLC, 25 m, 14 kg	12	1	0
5	XRG, 30 m, 12 kg	9	1	0
6	XLC, 30 m, 16 kg	10	3	0
Wild boar #				
1	XRG, 35 m, 45 kg	10	6	1
2	XRG, 40 m, 69 kg	15	1	0
Pig # (shoulder)				
1	BY, 40 m	12	3	1
2	XRG, 40 m	12	0	Nt

Nt: not tested

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The supernatants were combined, filtered through folded filter paper into Erlenmeyer flasks, and water was largely boiled off at a heating block. Then, 50 ml HNO_3 were added. After the formation of NO_x ceased, 2.5 ml of 30 % H_2O_2 were slowly added four times, which resulted in vigorous gas formation and colour change from brown to faintly yellow. Finally, the liquid was made up to 50 ml with distilled water.

The undigested residues remaining in the folded filter papers were combined with the sediment in the centrifuge bottles (representing the undigested and thus unavailable meat content) and were transferred to 1 liter glass bottles; 150 ml HNO_3 were added, and bottles were placed on a heating block at 80 °C to 110 °C. After about 2 hours, 5 ml of 30 % H_2O_2 were slowly added. Finally, the solution was made up to 100 ml with distilled water.

Determination of elemental composition of meat portions

Metal contents (Al, Cr, Cu, Fe, Ni, Pb, Zn) were determined by ICP-OES (Perkin-Elmer Optima 3000 XL; Perkin Elmer, USA), according to a protocol validated for meat and offal by Sager (2005a). Each run contained 2 blanks in appropriate dilutions and results for blanks were subtracted from results of actual samples. Dilutions were up from 1/20 to 1/10000, depending on the content of the element in the digest. In less diluted solutions, spectral interferences from the Cu-matrix were noted on the Cr-205, P-214, Na-330, and V-292 emission lines. Results are given as mg per 90 g portion.

Selection of studied metals was based on data on composition of the rifle bullets involved (Irschik et al., 2014; Paulsen et al., 2015).

Due to the limited number of samples and the preliminary nature of the findings, no statistical analysis was conducted.

Results and discussion

Characterization of samples

Dense particles were visible in all radiographs from wild game and in all three pork shoulders. After trimming and portioning of the meat (total 108 portions), the overall fraction of meat portions containing dense particles was 30/108 (28 %), but varied from 1/15 (6.7 %) to 9/13 (69.2 %) in game, and from 0/12 to 3/12 (25 %) in pork (Tab. 1). The dense particles could be metal or bone fragments. The latter was the most common cause, since all X-rays from game bodies and from pork shoulders demonstrated fractured and crushed bones, in particular scapula or humerus and ribs. Metal particles would be expected to be released from fragmenting bullets. BY and XRG should produce few, if any, fragments, and in XLC, fragmentation is unlikely. Admittedly, the actual behaviour of a rifle bullet is depending not only on construction and the target-tissue, but also velocity at impact (Gremse et al., 2014).

Metal contents in the digested and in the undigested fraction

Metal contents (median and maximum) in roe deer meat portions (digested and undigested fraction) are displayed in Table 2; Tables 3 and 4 give results for wild boar and pork respectively. Since no portions with dense particles were present in pork/XRG bullet, no data are reported. It must be noted that the liquid fraction obtained in this experiment is composed of the (1) "natural" metal content of meat, plus (2) the metal content in gastric and duodenal liquid simulants, and – in case that metal fragments are present in the meat portion –, in addition (3) the amount of metal released from bullet fragments during simulated digestion (Paulsen et al., 2015) as well as (4) the amounts of metal released from the embedded metal fragment into the surrounding meat during storage and processing (Irschik et al., 2014; Schuhmann-Irschik et al., 2015). From Tables 2–4, it can be seen that the median metal contents in controls are very similar to those in meat packs with presumed metal contamination. Also, median total metal contents (i. e. the sums of metal contents in the digested plus undigested fraction plus metal content in simulated gastric/duodenal juice; adjusted to mg/kg) were comparable to reference values reported by Sager (2005b) and Chan (1995).

Meat portions with evidence of the presence of bullet particles

Among the 108 meat portions under study, 30 packs contained radiodense particles. There were however, only 3 packs (2.8 %) with high metal content indicative for the presence of bullet fragments. In these three packs, the pattern of higher metal contents was consistent with information about the elemental composition of the bullets (Irschik et al., 2014), e. g. high aluminum content was found in meat of an aminal killed with a bullet containing an aluminium tip. Yet, only a very limited quantity of metals was present in the digestive fluids, whereas the major fraction was in the – undigested – residue, as shown in Table 5.

TABLE 2: *Metal contents in the digested and undigested fraction of roe deer meat portions, median and maximum value (µg per 90 g portion).*

	Control (n = 6)		XLC (n = 10)		XRG (n = 10)	
	Dig.	Residue	Dig.	Residue	Dig.	Residue
Al	29 (152)	521 (1259)	56 (87)	575 (863)	35 (83)	571 (68614)
Cr	2 (3)	3 (5)	3 (5)	3 (6)	4 (4)	4 (7)
Cu	48 (90)	56 (75)	49 (60)	53 (85)	51 (54)	58 (102)
Fe	604 (875)	1344 (1756)	382 (556)	1247 (1351)	408 (816)	130 (1791)
Ni	9 (13)	22 (28)	5 (7)	3 (5)	5 (7)	3 (5)
Pb	<1 (9)	1 (10)	2 (13)	1 (10)	2 (10)	2 (10)
Zn	550 (721)	2246 (2781)	576 (920)	2281 (3382)	350 (755)	2311 (3090)

Dig.: digested (liquid) fraction

TABLE 3: *Metal contents in the digested and undigested fraction of wild boar meat portions, median and maximum value (µg per 90 g portion).*

	Control (n = 2)		XRG (n = 7)	
	Dig.	Residue	Dig.	Residue
Al	149 (182)	549 (575)	54 (72)	830 (1186)
Cr	4 (5)	3 (3)	3 (4)	3 (6)
Cu	45 (52)	75 (78)	42 (49)	100 (3236)
Fe	424 (476)	1598 (1675)	382 (433)	1470 (2173)
Ni	19 (25)	6 (6)	5 (9)	7 (10)
Pb	5 (9)	8 (8)	4 (13)	3 (3)
Zn	490 (551)	2193 (2297)	475 (782)	2880 (4052)

Dig.: digested (liquid) fraction

TABLE 4: *Metal contents in the digested and undigested fraction of pork portions, median and maximum value (µg per 90 g portion).*

	Control (n = 6)		BY (n = 3)	
	Dig.	Residue	Dig.	Residue
Al	34 (38)	521 (1259)	43 (64)	406 (452)
Cr	2 (3)	3 (5)	2 (3)	2 (3)
Cu	20 (23)	56 (75)	22 (30)	55 (5004)
Fe	157 (158)	1344 (1756)	141 (244)	517 (564)
Ni	8 (10)	22 (28)	7 (8)	4 (6)
Pb	<1 (1)	1 (10)	15 (28)	<1 (234)
Zn	436 (472)	960 (1075)	338 (605)	1390 (4349)

Dig.: digested (liquid) fraction

TABLE 5: *High metal contents indicative for bullet fragment contamination, ratio of metal in the dissolved to that in the undigested fraction (µg per 90 g meat portion).*

	Roe deer, XRG	Wild boar, XRG	Pork, BY
Al	60/68614*		
Cu		32/3236**	22/5004**
Pb			15/234**

*: attributable to the aluminium tip of the bullet; **: attributable to the body of the bullet

Conclusions, underlying assumptions and limitations of this study

In this study, we could demonstrate that in meat from animals killed by rifle shot radiodense particles can be expected, even when the meat is trimmed carefully. Only a small fraction of these portions produced from undamaged meat adjacent to the shot wound contained metal levels indicative for embedded bullets particles. This was not unexpected, since the bullet types under study had low tendency to fragment. But even in the meat portions with high metal contents, the bioavailable fraction of metals was in the range as for meat portions with low metal levels. Although the data originate from a small sample set and should be considered a preliminary finding, the approach of studying meat portions instead of small aliquots and the assessment, which fraction of metal particles will actually be available for absorption, has its merits, when metal contamination is inhomogeneous. In this case, it is entirely conceivable that a meat sample with metal contents exceeding legal limits or tolerable daily intake values would in practice not effectuate higher metal exposure to humans. Currently, few studies adress this issue in humans (Mateo et al., 2007) or animals (Thomas and McGill, 2008). Admittedly, the protocol for simulation of gastric/duodenal digestion will have an impact on the results. Further studies will consider this issue.

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Conflict of interest

The authors declare that no conflicts of interest exist.

References

Bundesinstitut für Risikobewertung (BfR) (2010): Bleibelastung von Wildbret durch Verwendung von Bleimunition bei der Jagd, Stellungnahme Nr. 040/2011 des BfR vom 3. Dezember 2010. Available at: www.bfr.bund.de/cm/343/bleibelastung-von-wildbret-durch-verwendung-von-bleimunition-bei-der-jagd.pdf

Chan W, Brown J, Lee SM, Buss DH (1996): Meat, poultry and game, Supplement to McCance & Widdowson's – The Composition of Foods. The Royal Society of Chemistry, London.

Dobrowolska A, Melosik M (2008): Bullet- derived lead in tissues of the wild boar *(Sus scrofa)* and red deer *(Cervus elaphus)*. Eur J. Wildl Res 54: 231–235.

Gremse F, Krone O, Thamm M, Kiessling F, Tolba RH, Rieger S, Gremse C (2014): Performance of lead-free versus lead-based hunting ammunition in ballistic soap. PloS One doi: 10.1371/journal.pone.0102015.

Hecht H (1984): Untersuchung der Kontamination des Wildbretes an Blei und anderen Spurenelementen durch Schrot und absplitternde und dadurch weit im Tierkörper streuende Blei- und Metallpartikel der modernen Hochleistungsgeschosse. DFG-Forschungsvorhaben Ha 517/23 u. Ha 517/27-1-2-3, Abschlußbericht 1984.

Hunt WG, Watson RT, Oaks JL, Parish CN, Burnham KK, Tucker RL, Belthoff JR, Hart G, Zhang B (2009): Lead bullet fragments in venison from rifle-killed deer: potential for human dietary exposure. PLoS One doi:10.1371/journal.pone.0005330.

Fisher IJ, Pain DJ, Thomas VG (2006): A review of lead poisoning from ammunition sources in terrestrial birds. Biol Conserv 131: 421–432.

Irschik I, Bauer F, Sager M, Paulsen P (2013): Copper residues in meat from wild artiodactyls hunted with two types of rifle bullets manufactured from copper. Eur J Wildl Res 59: 129–136.

Irschik I, Wanek C, Bauer F, Sager M, Paulsen P (2014): Composition of bullets used for hunting and food safety considerations. In: Paulsen P, Bauer, A, Smulders, FJM (eds.), Trends in game meat hygiene: From forest to fork. Wageningen, Wageningen Academic Publishers, NL, 363–370.

Iqbal S, Blumenthal W, Kennedy C, Yip FY, Pickard S, Flanders WD, Loringer K, Kruger K, Caldwell KL, Brown MJ (2009): Hunting with lead: association between blood lead levels and wild game consumption. Env Res 109: 952–959.

Knott J, Gilbert J, Hoccom DG, Green RE (2010): Implications for wildlife and humans of dietary exposure to lead from fragments of lead rifle bullets in deer shot in the UK. Sci Total Env 409: 95–99.

Mateo R, Rodriguez-de la Cruz M, Vidal D, Reglero M, Camarero P (2007): Transfer of lead from shot pellets to game meat during cooking. Sci Total Env 372: 480–485.

Mateo R, Baos A R, Vidal D, Camarero P R, Martinez-Haro M, Taggart M A (2011): Bioaccessibility of Pb from ammunition in game meat is affected by cooking treatments. PLoS One 6 doi:10.1371/journal.pone.0015892.

Paulsen P, Sager M, Bauer F, Schuhmann-Irschik I (2015): Model studies for the release of metals from embedded rifle bullet fragments during simulated meat storage and food ingestion. Eur J Wildl Res 61: 629–633.

Sager M (2005a): Multi-Elementbestimmung in Fleisch, Leber und Nieren. Ernährung/Nutrition 29: 151–156.

Sager M (2005b): Aktuelle Elementgehalte in Fleisch, Leber und Nieren aus Österreich. Ernährung/Nutrition 29: 199–206.

Schuhmann-Irschik I, Sager M, Paulsen P, Tichy A, Bauer F (2015): Release of copper from embedded solid copper bullets into muscle and fat tissues of fallow deer *(Dama dama)*, roe deer *(Capreolus capreolus)* and wild boar *(Sus scrofa)* and effect of copper content on oxidative stability of heat-processed meat. Meat Sci 108: 21–27.

Thomas VD, McGill IR (2008): Dissolution of copper, tin, and iron from sintered tungsten-bronze spheres in a simulated avian gizzard, and an assessment of their potential toxicity to birds. Sci Total Env 394: 283–289.

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