Lead in game birds in Denmark: levels and sources

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Abstract: In June 2008, the National Food Agency contacted Bjarne Frost Vildt[1] against the background that the Danish surveillance of heavy metals in food (EU Directive 96/23 of 29 April 1996) had, for several years, shown elevated lead levels in game meat. These elevated levels exceeded the official threshold limits for food, with a significant prevalence in game bird species, in particular pheasant and, sporadically, venison.

As a result, Bjarne Frost Vildt submitted an action plan, including a campaign to raise awareness of the Danish regulations on lead shot, and the establishment of a research project to identify the source of lead in game meat. In July 2008, the Danish Academy of Hunting was tasked to design and carry out the investigation, in cooperation with the Veterinary Institute (*Technical University of Denmark*) and Food Region North (*Ministry of Food, Agriculture and Fisheries)*. The study was carried out from August 2008 to April 2009, and followed up in April 2010 and October 2011.

The study was based on sampling of a control group of 30 pheasants (*Phasianus colchicus*) on six private estates which had shown elevated lead levels in 2007. For this group, the local employees were instructed to be careful to ensure that the samples were obtained either with steel (non-lead) shot or without the use of firearms. However, at one estate the sample (N=5) was taken with bismuth shot. The lead levels of the control group were measured using the same methodology as the standard measurement (ICP-MS Agilent 7500i) and compared statistically with lead levels from the previous standard measurements. This showed markedly lower levels of lead (statistically significant), when compared to the 2007 standard, in birds that originated from the same districts. Based on x-ray and dissection, the number of "shot-in" pellets in the 2008 standard and the 2008 control groups was estimated and compared to the measured lead levels in the total group. A positive and statistically significant correlation was identified between the number of shot pellets found in pheasant and residue levels in the meat. The standard measurement carried out by Food Region North on pheasant sampled during the hunting seasons 2008/2009, 2009/2010 and 2010/2011, showed a decline in the prevalence of pheasants exceeding the official threshold limits, in comparison with previous years.

To supplement the data, lead levels were measured in meat of pheasants with two lead shot (3.2 mm) embedded. Further, lead was measured in pheasant meat penetrated by six lead shot (3.2 mm). To quantify the erosion impact of the preparation procedure (grinding), two lead and two bismuth shot were placed in pheasant meat before preparation and weight loss was calculated. The lead content of two bismuth shot from two different cartridges was also measured. Similar measurements were made on new generations of bismuth shot in January 2010.

Additionally, 1,434 gizzards from mainly pheasant (N=614) and mallard (*Anas platyrhynchos*) (N=656) were sampled, x-rayed, and dissected. Shot were categorized in shot types and origin ("shot-in" or "ingested").

It was concluded that the source of elevated lead levels is not contained in the food or the general environment of the birds, although ingestion of lead shot is a possible minor source. Different and independent factors indicate that the lead in the meat samples first and foremost originates from shot and fragments of shot situated in the breast muscles of the bird, and thus contaminating the sample for lead measurement. The contamination is accelerated by the method used for preparation of the sample (grinding). It was concluded that the elevated lead levels originate from the continued and illegal use of lead shot for hunting, and also from bismuth shot in which lead was found to be a contaminator. The decline in prevalence of pheasant meat exceeding the threshold limit during the hunting seasons 2008/2009 and 2009/2010 may be driven by three

[1] The biggest Danish butchery for game meat

different reasons: reduced illegal use of lead shot due to the campaign initiated in 2008; reduced concentration of lead in bismuth shot (2009/2010) due to the conclusions of this study; and/or reluctance to deliver pheasants to official slaughterhouses by districts being aware of illegal use of lead shot by hunters, and being aware of the study and the general attention being given.

1. Background

1.1 The study

Since the year 2000, lead content of game meat has been monitored in Denmark according to EU Directive 96/23. 29 April 1996. The monitoring is undertaken by the Ministry of Food (Northern Region). The game species involved are primarily pheasant (*Phasianus colchicus*), mallard (*Anas platyrhynchos*) and wood pigeon (*Colomba palumbus*), and lead levels exceeding 0.1 mgPb/kg (corresponding to the action threshold for poultry[2]) have been found repeatedly in all species.

Table 1 shows the results of the analysis of pheasant for the years 2000–2009 (unit: mg Pb/kg), showing that for some samples there is a very large excess over the action limit of 0.1 mg Pb/kg.

Year	N	Min	Max	Mean	Deviation	Median
2000	28	0	**0.556**	0,056	0.111	0.011
2001	26	0	**119**	**4.66**	23.3	0.0043
2002	26	0.00073	**7.6**	**0.508**	1.56	0.006
2003	25	0.00046	**0.165**	0.023	0.0464	0.0028
2004	46	0	**0.432**	0.0448	0.0951	0.0051
2005	70	0.0006	**10.9**	**0.449**	1.44	0.023
2006	20	0.0005	**7.97**	**0.601**	1.82	0.0109
2007	89	0.0003	**81.3**	**1.27**	8.93	0.0157
2008	56	0.0001	**42.2**	**0.95**	5.65	0.027
2009	30	0.0014	**3.9**	**0.16**	0.71	0.0057

Table 1. Content of lead in pheasants 2000–2009. Source: Food Northern Region Reports, years 2000–2007.> 0.1 mg Pb/kg is shown in ***bold.***

On this basis, the Food Authority approached *Bjarne Frost Vildt*, who submitted an action plan involving practical actions to be taken in the hunting season 2008/2009, including the establishment of a research project to identify the source of lead in game meat. In July 2008, the Danish Academy of Hunting was tasked to design and carry out the investigation in cooperation with the Veterinary Institute (*The Danish Technical University*) and Food Region North (*Ministry of Foods*). The study was carried out during August 2008 to April 2009, and followed up in March 2010.

1.2 Lead sources

Lead in birds could theoretically be admitted through the bird's digestive system. The source may be contaminated food or ingestion of lead containing shot scattered by hunting in the past or the same hunting season. The density of shot in the environment is quite large. In some hunting districts, 50,000 rounds or more may be fired annually, resulting in dispersion of 1.5 tonnes, or up to 10 million shot, being deposited in the environment and often in the birds' feeding grounds. British studies have shown that 3% of pheasants (N=437) from 32 properties have shot deposited in their gizzards (Butler *et al* 2005).

The source of the lead can also be shot which has been injected into the bird either from the lethal shot or from previous wounding. Pain *et al* (2010) show that lead gunshot undergoes sufficient fragmentation on

[2] For the years 2000 to 2006, the national threshold values for poultry meat was 0.3 mg Pb/kg. In 2007, this was replaced by an "action limit" of 0.1 mg Pb/kg. In 2008 this was changed to 0.5 mg Pb /kg. When used in this study, the term "action limit" means 0.1 mg Pb/kg.

impact with gamebirds for lead fragments to cause contamination of their meat. Johansen *et al* similarly showed that lead concentration is very high in meat of eiders killed with lead shot, and about 44 times higher than in drowned eiders. For some species, e.g. common eider (*Somateria mollissima*) and pink-footed goose (*Anser brachyrhynchus)*, studies have shown that between one quarter and one third have shot in the body, resulting from wounding. Similarly, the proportion of wounded birds was examined for pheasant (6%), mallard (15%) and wood pigeon (3%) (Noer *et al* 2006). Lead shot, which is embedded in the tissue by wounding, will normally be encapsulated, and lead from such shot is not spread in the tissue/body (Sanderson *et al* 1998).

A fundamental theory behind the study was that the source of lead in the game is lead shot from hunting: either shot that is ingested by the birds, or that has been injected as a result of the killing shot or previous wounding. Both possibilities should be considered given the background that, since 1996, the use of lead shot has been prohibited for hunting in Denmark, while a number of alternative materials have been introduced. These materials, for example bismuth and tin, may contain lead and provide a source of the measured elevated lead levels in game meat.

Previously published studies suggest that the shot in the environment becomes inaccessible to food-seeking birds relatively quickly. For example, Anderson (2000) shows that shot ingested by waterfowl along one of the central flyways of North America (Mississippi Flyway), was found to be mainly non-lead shot within a few years of the ban on the use of lead shot (1991). Similarly, it can be assumed that shot spread from hunting in an agricultural landscape, will relatively quickly be unavailable for pheasants and other bird species feeding in this ecosystem.

If the source of elevated levels of lead in Danish birds is shot ingested from their habitat, it is therefore likely that such shot has been spread recently rather than before the ban on lead shot, which came into force in agricultural habitats in 1993 and in forests in 1996. If the source of lead is "shot-in" lead shot, this is a clear proof of the continued use and therefore illegal use of lead shot. An unpublished study by the Danish Academy of Hunting in 2007 showed that “shot-in” shot in six out of 36 pheasants and mallard gizzards was lead shot. This result suggests the continued use of lead shot for hunting in Denmark.

British surveys (Food Standards Agency 2007) describe measurement of lead content in “some” samples of pheasant, recording a mean of 0.23 mg Pb/kg and a maximum of 1.63 mg Pb/kg. It was concluded that one possible source was non-visible fragments of lead shot in the samples. There is no prohibition on the use of lead shot for pheasant hunting in Britain, but on the basis of the survey, authorities recommended the use of other types of shot.

1.3 Existing Danish data on lead levels

Food Northern Region has made detailed results for the years 2003–2009 available. The studies include material from pheasant, mallard, wood pigeon, doves, deer and other animals (ostrich, cattle, pig, chicken, sheep and horse). During 2003–2007, a total of 1,246 samples were taken, of which 483 samples originated from wild animals, including 408 from the three bird species: pheasant, mallard and wood pigeon, with 250 of those being from pheasant. Out of the total of 1,246 samples, 58 exceeded the threshold lead limits set for each of the actual years. 57 of these were samples from wild animals, 51 from birds, and 38 from pheasant. During the years 2005–2007, the prevalence of pheasant with elevated lead levels were 20% or more.

2. Objective

The study aimed to identify the source of elevated levels of lead found repeatedly in game birds.

3. Methods and materials

The study employed four methods: 1. continued and expanded measurement of lead in game birds submitted to the slaughterhouse *Bjarne Frost Vildt* (mainly pheasants), combined with measurement of a control group; 2. analysis of shot in body and gizzards of the same pheasants and other birds; 3. measurement of contamination of lead shot embedded in samples and passing through game meat; and 4. measurement of lead in bismuth and the impact of erosion of shot during the grinding preparation.

3.1 Trace element analysis 2008

Measurement of trace element contents has been implemented as an extension and continuation of the measurements already conducted by Food Northern Region (Laboratory of Lystrup). Homogenized samples were dissolved in nitric acid and the trace element content was measured using ICP-MS Agilent 7500i (ICP-MS).

During the hunting season in 2008/2009, the laboratory team collected samples of 56 pheasants, 15 mallard, nine wood pigeon and 20 deer. This standard sampling followed the same procedure as previous years, and independently of this study. Preparation was undertaken by isolating 100–200 g breast meat, which was homogenized by grinding. 0.5 g was taken for the ICP-MS measurement.

As part of the study, this sample was supplied with 46 pheasants from six districts, which provided pheasants with elevated lead levels in 2007. The control group was sampled by the district staff at the turn of 2008/2009, and it was stressed that the selection should be independent of general hunts on the properties, using steel shot or, possibly, without the use of firearms. All birds were X-rayed. Trace element measurements following the standard procedure were made on five birds from the six properties, totaling 30 birds. The birds were subsequently dissected, and a total of 15 shot were isolated. X-ray photos were analyzed and shot counted and localized.

3.2 Analysis of gizzards

A total of 1,434 gizzards, including 614 from pheasant and 656 from mallard, and a smaller number from other dabbling ducks, geese and wood pigeon, were collected through Bjarne Frost Vildt, directly from hunting districts, from individual hunters and from birds used for monitoring of trace element content. Most gizzards were collected in Eastern Denmark. However, most of the mallard gizzards were collected in Jutland. The other dabbling ducks and geese were collected in West Jutland. The collected gizzards are not necessarily representative of all districts or country-wide in terms of incidence of shot materials used. Regarding pheasant gizzards from Bjarne Frost Vildt, the supply of game birds may have been affected by the pre-hunting season in 2008, where districts were aware of the general focus on lead shot, including this project. Gizzards with lead shot are therefore estimated to be under-represented, and the measured volume must be seen as a minimum level.

All gizzards were X-rayed and, samples containing shot were subsequently dissected and sorted according to the following procedure. Gizzards were inspected externally for shot holes, which were, wherever possible, marked with needle (Figure 1), after which the gizzard was opened and the contents washed out into a tray. The gizzards were then inspected for the shot holes on the inside, and shot was isolated in the gizzard content or dissected out of muscles. The shot was subsequently photographed, inspected and categorized using the following system (in which only non-magnetic and non-fragmenting shot were investigated for density and melting point). With this method of analysis, shot was categorized by reference to the origin (“shot-in” or “ingested”) and partly in relation to types of material. In each case it was not possible to determine whether the shot was shot-in or ingested. These are indicated as “?”.

A. Located in the muscle, fragmented/deformed (checked on X-ray photo), shot holes: shot shot-in.
B. Located in grit, small (<2 mm) worn/almond-shaped (checked on X-ray photo), polished, no shot holes: shot ingested.

1. Magnetic: Steel or Hevishot products.
2. Fragments: Bismuth
3. Dark material, high density, chewable and combustible: tungsten matrix products.
4. Dark material, high density, melting point of 280^o C: lead
5. Bright material, low specific gravity, melting point below 280^o C: tin.

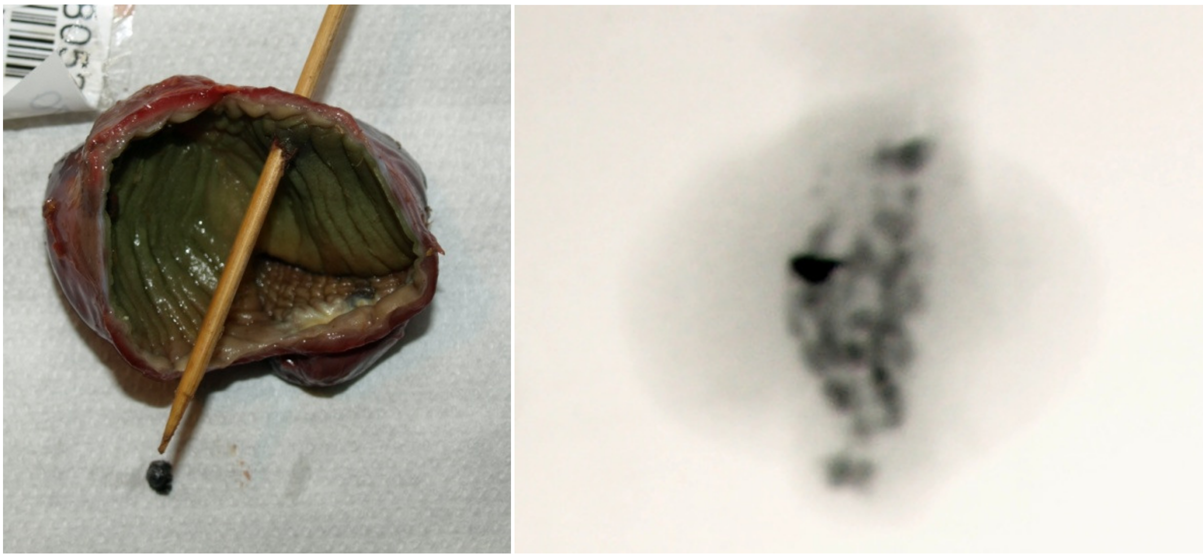

Figure 1. Gizzard of pigeon injected with lead shot. To the right is an X-ray photo of same gizzard, in which shots are seen with a deformation, which is typical of lead shot that hit grit.

3.3 Contamination by lead shot

To measure the level of contamination by lead shot embedded in the test sample and therefore ground in the preparation procedure, two 3.2 mm lead shot were placed in 150 g pheasant breast meat. Similarly, to measure the contamination of lead shot passing through the sample, a similar piece of pheasant breast meat was penetrated by six 3.2 mm lead shot, fired by a specially designed air gun. A control was constructed using meat from the same pheasant. The three samples were prepared uniformly and measured by ICP-MS.

3.4 Analysis of lead in bismuth shot and erosion

To measure content of lead in bismuth shot, three samples were used: one from six shot isolated from pheasants from one of the control districts; one from five shot isolated from an ordinary bismuth shot cartridge bought in a typical Danish retail gun store in 2009; and one similar bought in 2010. The samples were pulverized by use of non-leaded tools and afterwards submitted to ICP-MS measurement.

To measure the impact of grinding procedure on shot, two bismuth and two lead shot were photographed and weighed and placed in a standard media of pheasant meet and exposed to a standard grinding procedure. Afterwards the remaining shot and visible fragments were isolated, photographed and weighed.

4. Results

4.1 Trace element analysis and control

The results of the standard lead content measurement of 56 pheasants, 15 mallard and nine wood pigeons is shown in Table 2. In terms of elevated lead levels, pheasant does not differ from the corresponding figures for 2000–2007 (Table 1).

	N	Min	Max	Mean	Deviation	Median
Pheasants	56	0.0001	42.2	0.950	5.650	0.027
Mallard	15	0.0046	1.27	0.110	0.320	0.022
Wood pigeon	9	0.0010	0.26	0.045	0.084	0.010

Table 2. The outcome of the Regional Food standard measurements in 2008.Unit: mg Pb/kg.

Against this, the result of the measurement of the control group of 30 pheasant are as follows. 25 had a lead concentration below the detection limit of 0.0033 mg Pb/kg. The lead content of the remaining five shot were: 0.0052, 0.0066, 0.0147, 0.0149, and 0.83 mg Pb/kg. In this sample there are no reported values of results below the detection limit (0.0033 mgPb/kg) and therefore there no numerical comparison can be made of the mean or median with the standard sample. However, a comparison of the number of results below 0.0033 mg Pb/kg in the two groups showed that eight (14.3%) out of 56 standard measurements, and 25 (83.3%) out of 30 in the control group, were below this value. The median of the five highest values in the standard group is 2.23 mg Pb/kg, while in the control group it was only 0.0147 mg Pb/kg. A statistical analysis of the material (non-parametric test (Wildcoxon Rangsum)) showed this difference to be statistically significant (p<0.0001). Against this background, it is concluded that the lead concentration in the standard group is significantly higher than in the control group.

Based on X-ray and partly on dissection it was possible to estimate the number of shot in the breast muscles of 73 birds (49 pheasants, 15 mallards and nine wood pigeons). Furthermore, a partial determination of shot types was made. X-ray photos cannot be used for a complete determination, but in cases where there is evidence of fragmented shot, they are categorized as bismuth shot. Shot deformed without being fragmented is likely to be lead or tin shot. Large and round shot are assumed to be steel shot. Localization and identification of shot by the use of X-ray photographs is a relatively inaccurate method. Hence, numbers of shot in the breast muscles cannot be taken as absolute figures, but rather as an index.

Dissection was only undertaken on the control group, since the remains of birds from standard samples were not preserved. No full dissection of birds from the control group was conducted, this being a very time consuming procedure, but 15 shot were isolated, of which two were bismuth (both from property # 4) and the rest, steel (4 mm) (all from the other properties). There is thus no indication that the control group was sampled using lead shot or had lead shot injected.

The lead levels and the number of shot in the breast muscles were correlated for 42 out of the 73 birds, as data below the detection limit were omitted for 31 birds, of which none contained shot. For the data above the detection limit, we used a logarithmic transformation of lead levels, and a regression on both the number of lead shot and the square of the number (Figure 2). The residuals of this model were confirmed by Kolmogorov–Smirnov testing for normality (p> 0.1). The p-value for the effect of the number of shot (LR-test, F-test, usual multiple regression) on lead level is p <0.0001. Accordingly, we concluded a statistically significant increase in lead levels was related to the amount of shot.

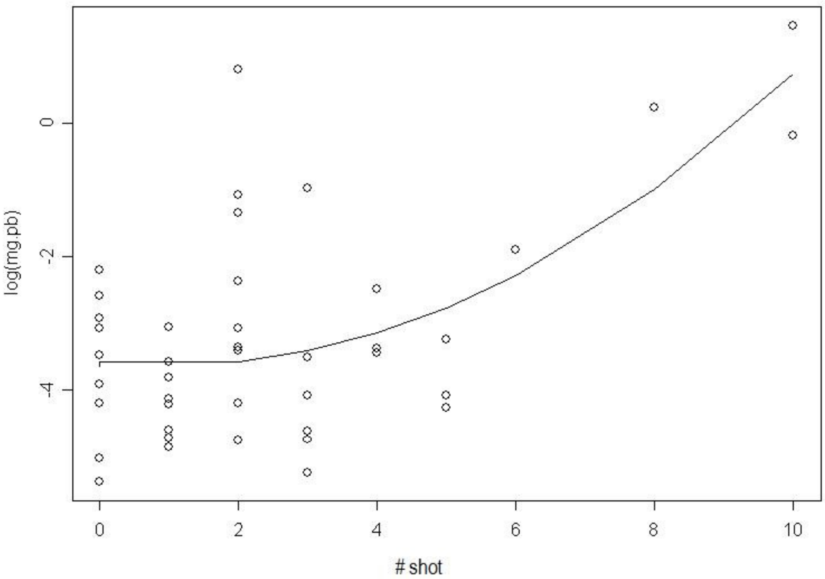

Figure 2. The correlation between the estimated number of shot in the thoracic muscles and the measured lead levels (logarithmic) in mg Pb/kg.

4.2 Shot in gizzards

Out of 1434 gizzards, using radiography, we detected shot in 296 (20.6%). From 292 of these, we isolated 449 pieces of shot (whole or fragmented) and categorized them by material and origin (ingested or shot-in). This is illustrated in Table 3 (all species), where the upper part indicates the distribution of all the shot, and the bottom shows the distribution of gizzards containing at least one shot. In several cases, shot of more than one type is found in the same gizzard, hence the difference between the number of investigated gizzard and totals. Tables 4 and 5 give the corresponding figures for pheasant and mallard, respectively.

Shot	**Ingested**	**Shot-in**	**?**	**Total**
Bismuth	144	85	2	231
Steel	55	100	2	157
Lead	27	25	4	56
Tin	0	5	0	5
Total	*226*	*215*	*8*	*449*
Gizzards with shot	**Ingested**	**Shot-in**	**?**	**Total**
Bismuth	56	74	2	132
Steel	28	83	2	113
Lead	18	22	4	44
Tin	0	3	0	3
Total	*102*	*182*	*8*	*292*

Table 3. Distribution of 449 shot taken from 263 of the 1434 collected gizzards. Top: all shot; bottom: distribution of gizzards with at least one shot.

Shot	**Ingested**	**Shot**	**?**	**Total**
Bismuth	84	40	1	125
Steel	27	26	0	53
Lead	17	15	1	33
Tin	0	5	0	5
Total	128	86	2	216
Gizzards with shot	**Ingested**	**Shot**	**?**	**Total**
Bismuth	24	38	1	63
Steel	7	24	0	31
Lead	10	12	1	23
Tin	0	3	0	3
Total	41	77	2	120

Table 4. Distribution of 216 shot taken from 108 of the 614 collected pheasant gizzards. Top: all shot; bottom: distribution of gizzards with at least one shot.

Shot	**Ingested**	**Shot**	**?**	**Total**
Bismuth	60	43	1	104
Steel	26	64	2	92
Lead	10	9	2	21
Tin	0	0	0	0
Total	96	116	5	217
Gizzards with shot	**Ingested**	**Shot**	**?**	**Total**
Bismuth	32	34	1	67
Steel	19	51	2	72
Lead	8	9	2	19
Tin	0	0	0	0
Total	59	94	5	158

Table 5. Distribution of 217 shot taken from 141 of the 656 collected mallard gizzards. Top: all shot; bottom: distribution of gizzards with at least one shot.

In one gizzard we found 36 ingested shot (11 bismuth, 4 lead, and 21 steel) (Figure 3).

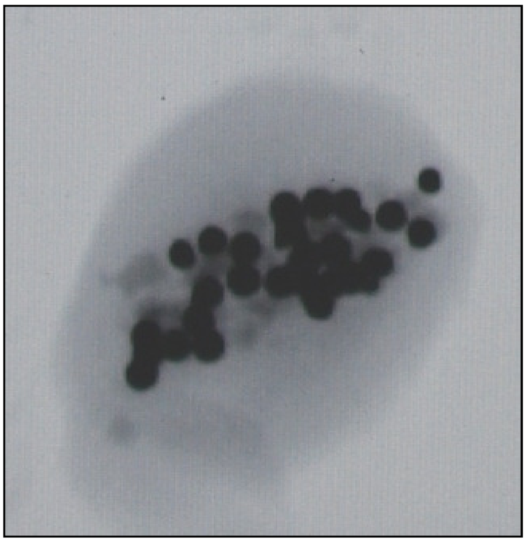

Figure 3. Pheasant gizzard with 36 ingested shot.

Figure 4 shows the overall distribution of ingested shot per gizzard, broken down into bismuth, steel and lead.

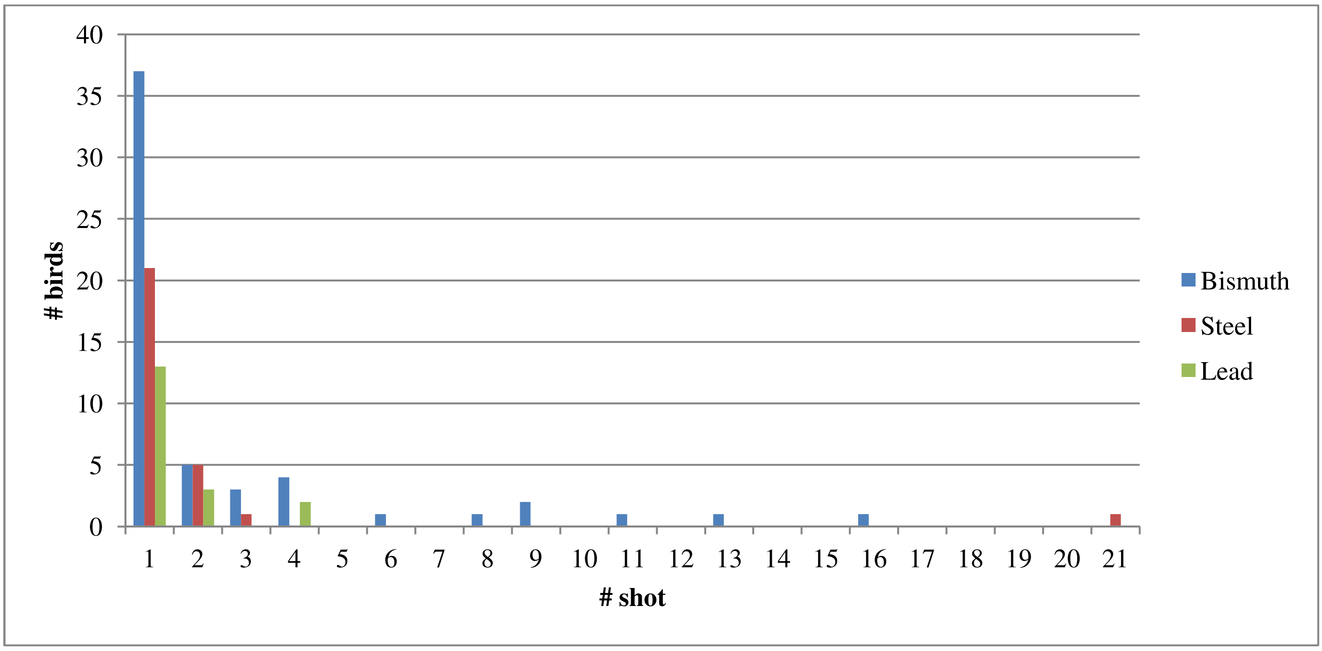

Figure 4. Breakdown of all ingested shot of bismuth, steel and lead (N=226).

4.3 Contamination from lead shot

The level of lead in the piece of pheasant breast meat with two embedded lead shot was 810 mg Pb/kg, indicating a very large contamination by shot situated in the sample and ground during the preparation procedure. The level in the sample that was penetrated by six lead shot was 0.122 mg Pb/kg, thus slightly above the threshold limit. The level in the control measurement was below 0.0033 mg Pb/kg.

4.4 Lead in bismuth and erosion by grinding

The level of lead in the three samples of bismuth shot is shown in Table 6. There are slight differences.

Bismuth shot sample	Lead level (mg Pb/kg)
Dissected from pheasant	6800
Bismuth shot retailed March 2009	3150
Bismuth shot retailed January 2010	950

Table 6. Lead levels in three samples of bismuth shot.

The erosion of bismuth and lead shot in the laboratory grinding preparation of samples is shown in Table 7. The grinding preparation causes fragmentation of bismuth shot, while the surface of lead shot becomes rough (Figure 5). These results indicate clearly the level of contamination that occurs during preparation of samples for the whole or fragmented shot.

Shot	Weight before (mg)	Weight after (mg)	Loss	%
Bi 1	146.9	133.3	13.6	9.3
Bi 2	132.6	101.1	31.5	23.8
Pb 1	127.6	123.6	4	3.1
Pb 2	152.1	149.7	2.4	1.6

Table 7. Erosion of two bismuth and two lead shot by preparation of samples made using the standard method of The Food Region.

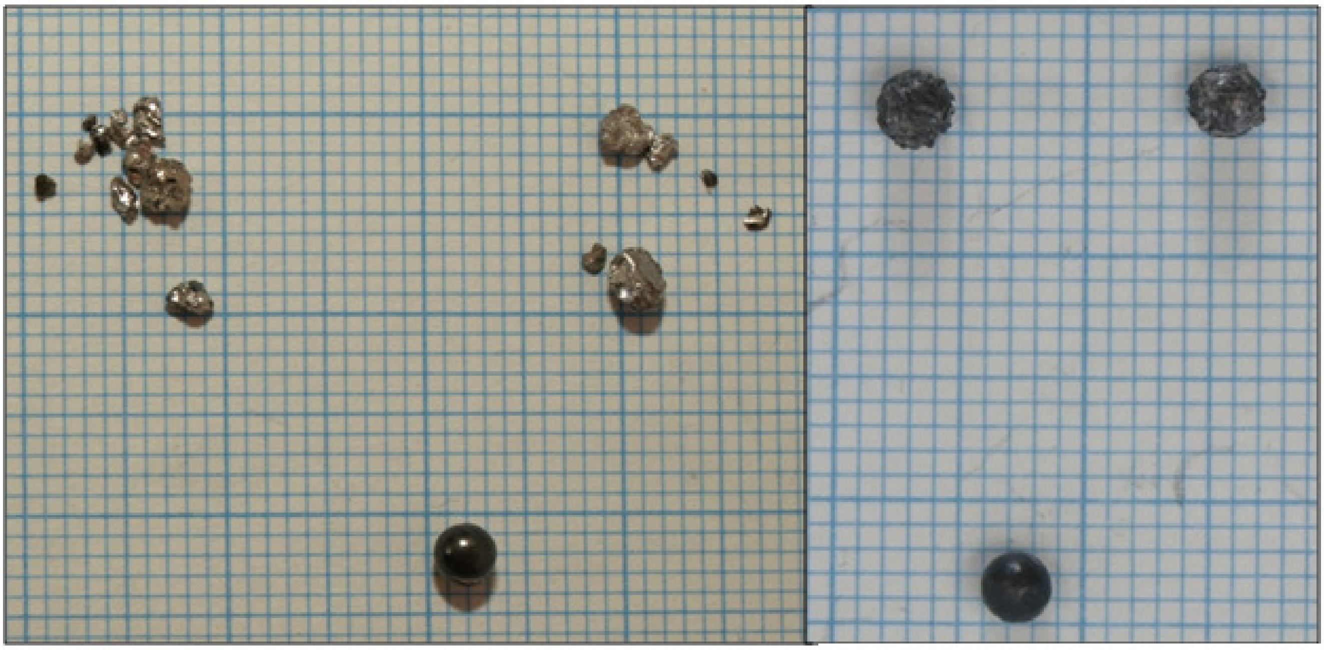

Figure 5. Top: shot after preparation of the sample (grinding). Bottom: shot before preparation of the sample. Left: bismuth. Right: Lead.

5. Discussion

5.1 Lead in feed and food

One theory is that the measured lead levels in game meat originate from the rearing phase, or the period immediately after release. Sewage sludge has been suggested as a possible source. However, there are several factors in the study, which suggests that the source is *not* in the wider environment. Firstly, the high levels are found in species that typically are reared and released for shooting, mainly pheasant and mallard, and in other game species such as wood pigeon. The species differ significantly in terms of breeding habitat and foraging, and it is unlikely that these wild species are exposed to the same lead sources through feed or natural food items.

Another indication is that the elevated lead levels typically occur sporadically. A lead source in food would be expected to give more even levels, so that we would see more birds with slightly elevated lead levels and not, as in the present results, very variable levels in birds from the same area (indicated by the distribution and differences in mean and median in Table 1). Results from previous years (Food Region pers. comm.) show very variable levels, even between samples from the same bird, suggesting that the lead content is not uniformly distributed, as would be expected if the elevated levels of lead had been admitted through the digestive tract (also concluded in Scheuhammer 1998).

Thirdly, a comparison of the standard group from 2007 (N=89) and the control group in 2008 (N=30) (where the birds were all predominantly collected at properties with elevated lead levels in 2007), indicates that the lead source can be eliminated only by modifying the method of collection/instruction of shooters. Except for the five birds in the control group, which were shot with bismuth shot (property # 4), only one (4%) of the remaining 25 measurements was above the detection limit of 0.0033 mg Pb/kg. 77 out of 89 (80 %) birds from the standard measurement in 2007 was above this limit. This result does not exclude that lead sources may be in feed or natural food items. However, if this were a major source, one would expect the same high incidence of measurements in the control group in 2008.

5.2 Lead from ingested shot

As mentioned initially, it is known that both waterfowl and terrestrial species ingest shot by confusing them with food items or grit (e.g. Fisher *et al* 2006; Pain *et al* 2009). Through both field and laboratory studies is has been shown that ingestion of lead shot causes elevated levels of lead. For example, Schultz *et al* (2009) find significantly elevated levels of lead in the blood and liver of mourning doves *(Zenaida macroura)* which have ingested lead shot. There are also a number of similar studies of waterfowl, e.g. Longcore *et al* (1974), who find similar results in feeding studies with mallards. Daury (1993) concludes similarly by comparing American ducks from areas with and without hunting with lead shot. Custer *et al* (1984) find the same correlation in studies of American kestrel (*Falco sparverius*).

Ingested shot remaining in the gizzard will be eroded. The shape in this process is typically an "almond" form (see Figure 7), which is also described by e.g. Ringelman 1993. Shot of different materials decomposes at different rates (Mitchell 2001). Brewer (2003) shows that steel shot over a given period lose seven times more weight than tungsten-containing shot (Hevishot) during feeding trials with mallard. A snapshot of the distribution of shot types found in the gizzard does not therefore necessarily give an accurate picture of the ingestion rates or the availability in the birds' environment. Similarly, "toxic" shot may be underrepresented, as a certain amount of this type of shot (e.g.> 4 lead shot) will in most cases be lethal.

However, it is evident that the birds have ingested both bismuth, lead and steel shot, and that the recorded distribution probably reflects approximately the inclusion of shot. 6.7% of pheasant had ingested shot in their gizzards, including 3.9% bismuth shot, 1.6% lead and 1.1% steel shot. Consequently, it can be concluded that lead shot are in the birds' environment, which indicates continued use of lead shot for hunting, although we could not exclude that there is still shot available since the ban in 1996.

Neither tin nor tungsten shot were found, which is probably due to the limited use of these types of shot. For polymer-based tungsten shot, it is further known that they erode fairly quickly in a bird's gizzard (Mitchell 2001).

No ingested shot have been localized in the gizzard of birds that have been the subject of measurement of trace element content. Therefore, it has not been possible on this basis to relate the intake of shot to elevated lead levels, and therefore to confirm or exclude whether the ingestion of shot can be a source of the elevated lead levels. The link can be judged from the fact that 1.6% of the surveyed gizzards contained ingested lead shot. Breakdown by number of shot per gizzard is shown in Figure 6.

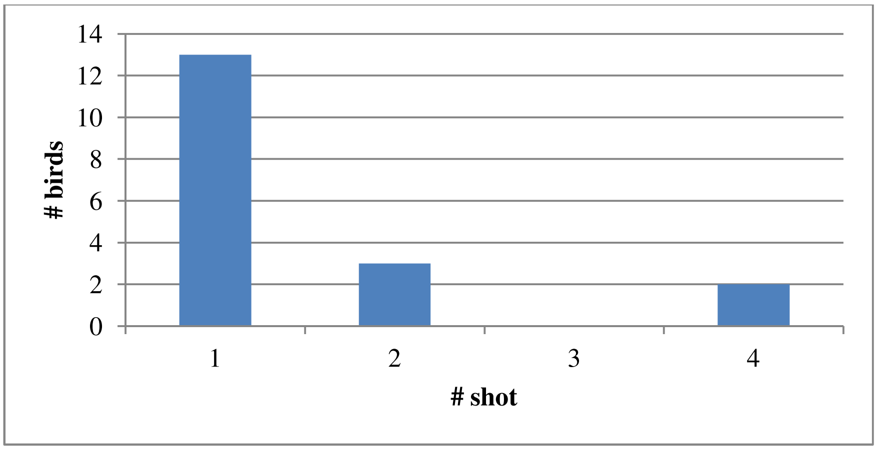

Figure 6. Distribution of number (N=19) of lead shot, eaten by pheasants.

As mentioned in the methodology section, the collected gizzards used in this study were not necessarily representative and the prevalence of ingested lead shot must probably be seen as a minimum figure. Yet, numbers do not seem to explain the prevalence of elevated lead levels (> 0.1 mg Pb/kg) which, in a number of years, was above 20%.

However, the measured levels of lead could well have arisen because the birds have ingested lead shot. A lead shot (diameter=3 mm) weighs approximately 140 mg. Assuming that such a shot erodes and is completely absorbed in a pheasant, which weighs 1 kg, the contamination is equivalent to a total lead content of 140 mg Pb/kg. Absorption of lead is not uniform in different organs and tissues. Concentration in muscles will be 30–50 times lower than concentrations in bone and kidney after both moderate and high lead intake in mallard (Longcore 1974). As the original size of the ingested lead shot is not known, their erosion in the gizzard cannot be calculated. The isolated lead shot weight ranges from ca. 120 mg to 20 mg, which indicates erosion similar to descriptions in previously published studies, which show an erosion of shot in the gizzard and consequent almond shaped shot (Figure 7).

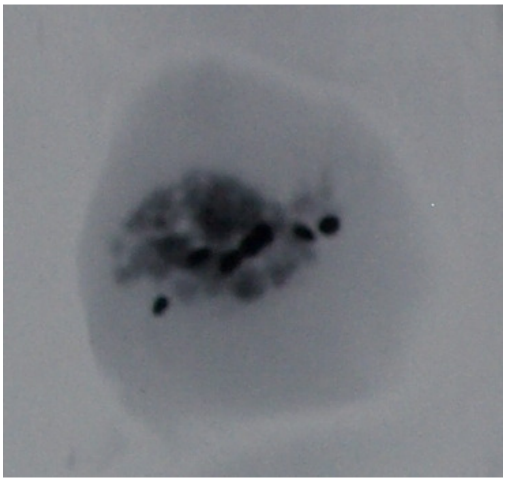

Figure 7. Pheasant gizzard with eroded lead shot. It is seen that shots lose their original round shape and adopt an "almond shape".

A 50 % erosion and absorption of 3 mm lead shot will leave a 70 mg Pb/kg/shot in a whole pheasant. Longcore (1974) shows levels of 1.2 to 1.7 mg Pp /kg of lead in breast muscle in mallard that had absorbed approximately 300 mg of lead. Fimreit (1984) showed that absorption of 129 and 159 mg lead from lead shot did not raise lead levels in breast muscle above 0.1 mg Pb/kg in grouse, whereas an absorption of 222 and 257 mg resulted in levels of 0.5 and 0.7 mg Pb/kg, respectively.

Even taking into account that the concentration in muscle is significantly lower than the average concentration, 1–2 lead shot can cause values that correspond to some of the elevated levels measured in the Danish studies, but is unlikely to be the reason for the sporadically occurring very *highly* elevated values seen in the standard monitoring of lead levels in game meat.

A similar assessment can be made for bismuth shot. Bismuth shot investigated in this study turned out to have a certain level of lead, measured at 0.7 % in shot isolated from hunted pheasants. It is known from literature that bismuth shot may contain lead. Jayasinghe (2004) shows the correlation between measurements of bismuth and lead in samples from birds with ingested bismuth shot. A 3 mm bismuth shot with a lead content of 0.7 %, eroded and absorbed by 50%, will cause a lead content of 0.8 mg Pb/kg/shot in a whole pheasant. Taking into account that the inclusion of lead in muscles is significantly lower than average uptake, as discussed above, ingested bismuth shot does not seem to contribute markedly to elevated lead levels, even in birds ingesting large amounts of shot. Breakdown of ingested bismuth shot in the analyzed pheasant gizzards is shown in Figure 8. In one case, we found 16 ingested bismuth shot in a gizzard, but were most frequently found as single shots.

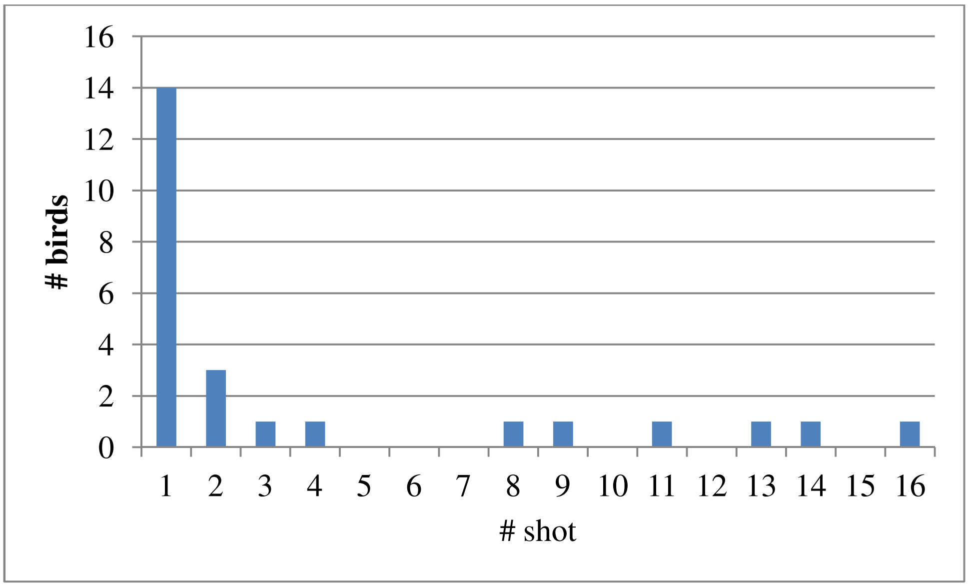

Figure 8. The breakdown of the number of bismuth shot (N=84) eaten by pheasants.

Based on this assessment and the measured levels of lead, it is estimated that ingested lead shot may be a source of some of the elevated values, but that ingested bismuth shot only slightly contributes to the elevated lead levels.

5.3 Lead from shot-in shot

As a result of the sampling and preparation methodology used in connection with ICP-MS measurements, there is a probability that whole shot, or fragments or traces of shot, are included in the sample. When the sample is grinded (homogenized), shot will erode/fragment, and the sample be contaminated. Homogenization is not complete and the outcome of the measurement will therefore depend on whether there is a high concentration of shot material in the 0.5 g meat, which is taken for further analysis. Inclusion of only 0.5 mg of lead (<0.4% of a shot) will affect the measurement results greatly (1,000 mg Pb/kg), as is seen in the controlled inclusion of two lead shot in a sample, resulting in 810 mg Pb/kg. Bismuth shot with a lead content of

1% also causes similar contamination of the sample. One fragment, for example of 5 mg bismuth shot, containing 0.05 mg of lead (1%), in a sample of 0.5 g, could lead to a lead level of 100 mg Pb/kg.

Whether the reason for the repeated elevated lead levels in standard monitoring is shot-in shot can be investigated on the basis of control measurements on the 30 pheasants taken out at the six properties where pheasants had significantly elevated lead levels in 2007. The results of this study suggest that the modification of the sampling methodology used for the control group causes a significant reduction in the measured lead levels, and that shot-in lead shot are a major source of elevated lead levels in birds.

Furthermore, the importance of shot-in shot can be discussed on the basis of dissected birds and gizzards. The entire study material, with the exception of a small number of the 30 pheasants in the control samples, was collected by using shotgun. This includes all 56 pheasants in the 2008 standard test that is applied during ordinary hunting.

Lead shot has been banned for use in hunting in Denmark since 1996, and a number of alternative shot materials have been marketed and are used today by Danish hunters. There are no precise statistics on the distribution of individual products, but steel shot has generally become popular for hunting in wetlands, and to some extent for upland game outside forests. Bismuth shot seems to be the dominant type of shot for hunting in the forests, where foresters in general do not allow the use of steel shot. Other products, such as tin and tungsten, may be available, but the price and other market-related reasons mean that their availability and use is sporadic.

When game is shot with a shotgun, it will be hit by a number of shot. At normal shooting distances, a bird of medium size, hit by the central parts of pattern, will typically be hit by 2–6 shot, some of which may pass through the bird, though the majority will be stopped by the muscles, organs, ligaments or bones. Figure 8 shows the distribution of shot in the 61 X-rayed hunted birds included in the study, and in which were recorded at least one shot.

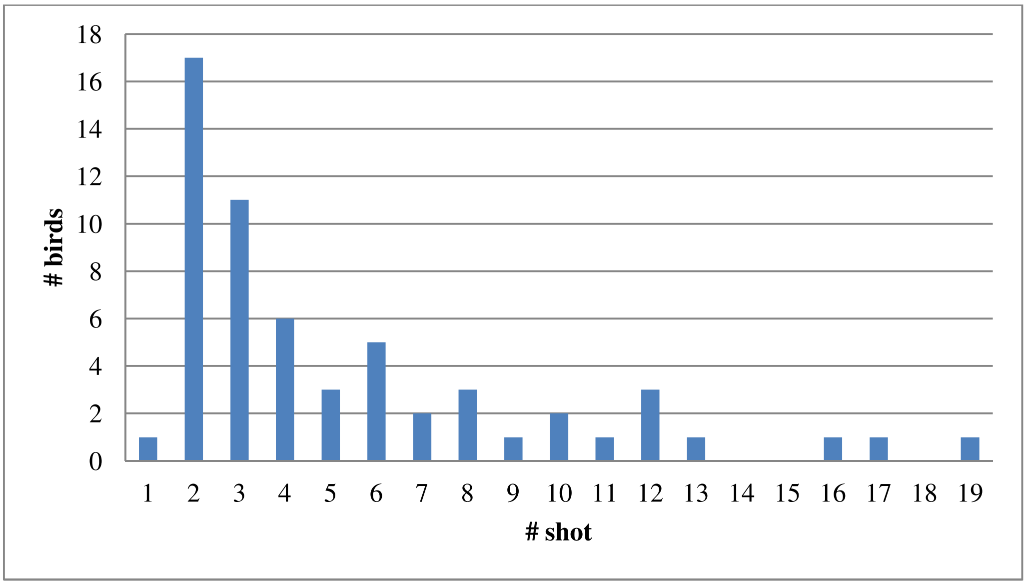

Figure 9. The distribution of shot in 61 birds bagged in the study (39 pheasants, 15 mallard and 7 wood pigeons).

An analysis of shot-in shot was made by radiography and dissection of all birds and collected gizzards (see Figure 1, 9 and 10). Radiography, as described in the methodology section, gave the possibility of distinguishing between types of shot: for example, in cases shot are clearly fragmented (bismuth). Similarly, shot type and material was determined by dissection and isolation of shot. In contrast to the analysis of ingested shot, the shot-in shot can be seen as an expression of the breakdown of the types used by hunters, although not necessarily representative of all districts and across the country.

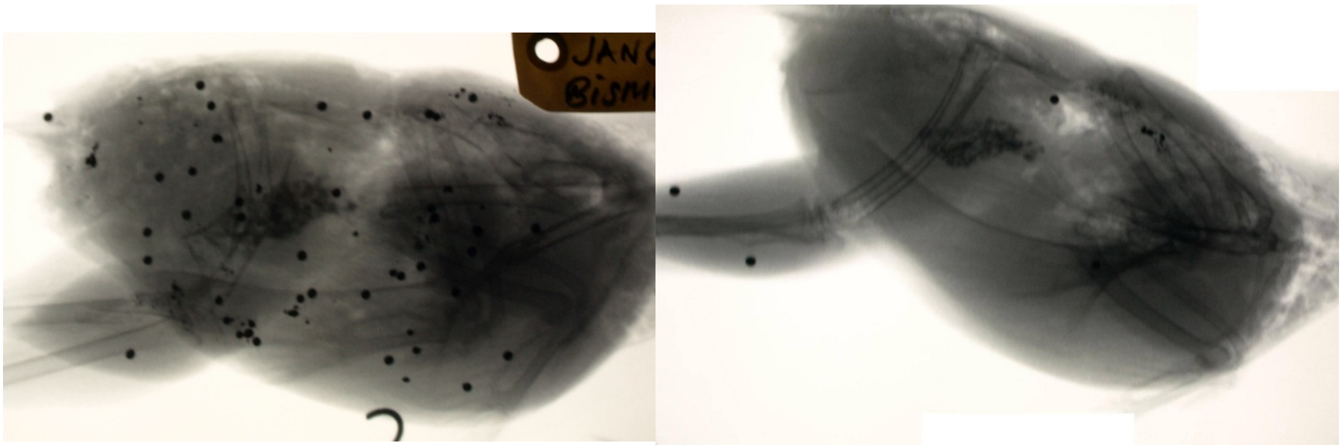

Figure 10. Pheasants with bismuth shot, which in some cases is clearly fragmented.

The results of this study show that, despite the ban on lead shot for hunting, lead shot is still used. Of the 77 pheasant gizzards with shot-in shot, 12 had lead shot (15.6%). For mallards there was lead shot in nine out of 94 gizzards, equivalent to 9.6%. Again, it must be emphasized that, because of the collection method, the findings are hardly representative of all districts or across the country, and are therefore probably minimum levels. In a somewhat lower sample-size from an unpublished study from 2007, six out of 36 shot-in shot in gizzards of pheasants and mallard were identified as lead shot, equivalent to 16.7%.

Because mallards are migratory birds, one might argue that deposits (lead) shot, could theoretically have come from wounding in other countries. Since most of the material were released mallards known to remain locally, it is not likely that wounding in other countries has contributed to the results.

The suspicion that lead shot is used for hunting in Denmark is confirmed by conversations with people who have direct contact with hunting management. Several districts have given information about discovery of empty cartridge cases, which corresponds to lead cartridges. The increased sales of lead cartridges in border regions in Germany and Sweden further suggest the illegal import and use of lead shot.

From the gizzard studies, it is estimated that between 10 and 20% of the hunted pheasants and mallard are taken with lead shot. In the somewhat lesser amount of material obtained for other species (N=6), there was included a single wood pigeon which was shot with lead shot. None of the gizzards from waterbirds, which originated from West Jutland (N=116), had shot-in (or ingested) lead shot.

An assessment of shot in whole birds suggests that about 33% of all shot are localized in the breast region, from which samples for lead measurements were taken. Experiments with the loading of lead shot in a sample showed that this can provide a very strong effect and shooting lead shot through pheasant meat also gives a measurable effect. This is further demonstrated by Johansen *et al* (2004) and Pain *et al* (2010).

Overall, these findings indicate that shot-in lead shot is a sporadically occurring source of contamination of samples for lead measurement. Deposited lead shot may be the cause of elevated values, which vary with repeated measurements of samples from the same bird. This corresponds to previously published results of U.S. studies. Scheuhammer (1998) concludes that the source of lead in breast muscles are lead shot or traces of lead shot, and that the values are highly dependent on where the sample is taken.

The calculations on the correlation between number of shot in the thoracic muscles and lead levels of the 71 birds also confirm the theory that the injected lead shot is a major source of elevated lead levels. Here was seen a clear correlation, probably due to the fact that the more shot that hit the bird, the greater the probability of shot or fragments of shot being included in the sample. As lead shot only fragments and erode to a limited extent, the probability increases that the material included in the sample is less. On the other hand, even small fragments of lead shot could result in a very high measurement. For bismuth shot, which may easily fragment on striking the bird, and particularly during the subsequent preparation of the sample, the likelihood of small pieces in the sample is relatively high. Quantitative estimates suggest that even relatively low lead levels in bismuth shot can explain some of the levels of lead seen in the standard monitoring.

The calculations are supplemented by a review of specific individual evaluations. In a single pheasant, which was part of the 2008 standard measurements, we measured a lead content of 42.2 mg Pb/kg, which is the highest value in the total material of 2008, and the second largest in the last four years of measurements on pheasant. X-ray photos of the bird shows one shot and fragments in the breast muscle, which appeared to indicate that bismuth or lead shot is the source. Since the bird is not preserved, it is no longer possible to use dissection to conduct an analysis of shot. Another pheasant, included in the control group from 2008, had no measurable lead content, but X-ray photograph indicates five shot in chest muscles. This bird was dissected, and all shot were found to be steel shot. Similarly, shot in birds with lead levels below the detection level were concluded to be non-lead or non-bismuth shot. Overall, we found that none of the birds (N=30), where the lead level was below the detection level, had lead or bismuth shot in the chest muscles.

The results suggest that the elevated lead levels in the standard monitoring predominantly derive from shot-in shot containing lead. Based on the available data, the importance of the respective lead and bismuth shot can only be assessed with caution. An evaluation of the prevalence of lead levels exceeding the 0.1 mg Pb/kg threshold limit over the years 2003–2010 is shown in Fig 11.

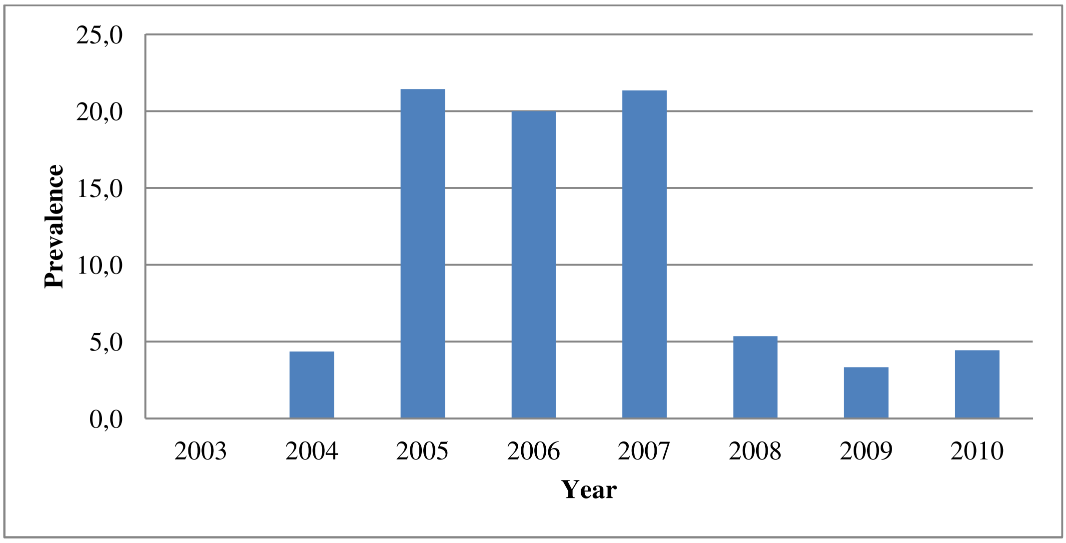

Figure 11. Prevalence of lead levels exceeding the 0.1 mg Pb/kg threshold limit for the years 2003–2010.

The very marked drop from 2007 to 2008 is most likely a consequence of the campaign to reduce the illegal use of lead shot that was launched before the 2008 hunting season. Also, awareness of this study may have played a role by making hunters either not use lead shot, or districts not delivering game to official slaughterhouses to avoid it being included in the standard measurements of lead content. As a consequence of the findings in this study, manufacturers in 2009 changed methodology and reduced the lead content of bismuth shot, which is also indicated in the measurement of the 2010-generation of bismuth shot, now containing 950 mg Pb/kg. However, this cannot explain the drop in prevalence of high lead levels from 2007 to 2008. We have focused specifically on the lead and bismuth shot, and not on tin shot, also believed to contain lead. Tin is part of bismuth shot and it cannot be excluded that lead in bismuth shot derives from added tin. There is no evidence that steel shot is a lead source.

6. Conclusion

There are several factors suggesting that the source of the measured excess of lead above the action limits is not found in bird food, natural food or in the surrounding environment. Firstly, excess lead is found in a variety of wildlife species with rather different breeding and foraging strategies. Secondly, control measurements on pheasants collected on properties that had significantly elevated lead levels in 2007, no longer showed elevated lead levels in 2008, although there had apparently been no changes in feed or environment. Thirdly, repeated measurements on samples from birds with high lead levels showed widely varying results and rarely confirmed the result of the initial measurement. This suggests that the lead content is not uniforly distributed throughout the tissue, as would be expected if the lead came through the bird's digestive system.

Similarly, there are a number of factors which suggest that the source of lead is associated with leaded ammunition. It is in this context demonstrated that bismuth shot contains lead, and the continued use lead shot for hunting means that lead shot is available for birds in the environment. The study did not indicate that ingestion of lead shot is the primary source of elevated lead levels.

Our study suggests that the major source of elevated lead levels is lead-containing shot that, during hunting, is shot into birds' muscle and thereby constitutes a very significant source of sporadically occurring elevated lead levels, when whole shot or fragments of metal from shot are included in the samples. Localization of shot indicates that about one third of the shot is located in the breast muscle from which the samples are collected. In experiments under controlled conditions, it is also shown that there may be erosion of shot during the laboratory preparation of samples, therefore explaining both the size and frequency of the measured lead levels.

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