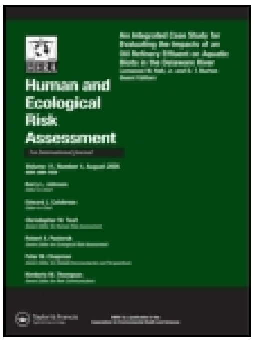

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Evidence of High Tolerance to Ecologically Relevant Lead Shot Pellet Exposures by an Upland Bird

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ABSTRACT

Upland birds that display grit ingestion behavior are potentially at risk of detrimental effects and death from lead poisoning at trap and skeet ranges and other areas where vast quantities of spent lead shot pellets abound. Because commonly cited force-feeding pellet exposure studies deviate from true field conditions, their results may not reflect true risks faced by upland birds. In particular, studies that use new shot pellets and administer more pellets than would be reasonably ingested, critically interfere with the understanding of actualized pellet exposures. In this study, northern bobwhites (*Colinus virginianus*), a frequent test species in shot pellet research, were dosed in an ecologically-relevant manner (*i.e.*, with spent shot and with no more than three pellets). Notably, the 56-day post-dosing observation period, during which a battery of physiological measures were recorded, exceeded that of related studies. Despite a sustained suppression of a lead poisoning indicator, the data suggest upland birds can withstand spent shot pellet exposures. Data detected a survivorship $\geq$95%, absence of illness, demonstrated tolerance for extremely high blood lead concentrations, and unaffected blood parameters. In conjunction with ecological considerations (*e.g.*, spatial scale and animal behavior), concern about bird population losses from the incidental ingestion of spent shot pellets is potentially overstated.

Key Words: upland birds, grit ingestion, lead shot, trap and skeet ranges, lead poisoning.

INTRODUCTION

The suggestion that birds incidentally ingest spent shot pellets at trap and skeet ranges has given rise to a palpable ecological risk assessment (ERA) issue of concern

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for more than a decade. Ground-dwelling avian species that typically ingest grit are reputedly at risk for ingesting lead-based pellets that approximate the mineral grit that birds occasionally consume (*i.e.*, small bits of rock or stone, 1–4 mm in diameter; Peddicord and LaKind 2000; Vyas *et al.* 2000, 2001; USEPA 2011). It is hypothesized that if a shot pellet is retained in the ventriculus where it will dissolve, a considerable blood lead concentration will develop. Lead toxicosis and ultimately lethality are anticipated to follow, allowing for significant population losses for some species.

The concern over significant bird population losses from pellet ingestion is seemingly fueled by two realities that affect the potential for excessive lead exposure: (1) there are approximately 9000 non-military target shooting ranges in the United States (USEPA 2001) and (2) vast accumulations of pellets are found in the fall zones of the ranges. In fact, the intensity of clay pigeon trap and skeet range use, and the discharge of up to 36 g of lead pellets per cartridge over a relatively small, well-defined area (Darling and Thomas 2003), can result in pellet densities of up to 3.7 billion per hectare (ha) in the top 7.5 cm of soil or sediment (Stansely *et al.* 1992). Hindering the understanding of the health effects associated with incidental pellet ingestion by upland birds, however, is the dearth of truly utilitarian pellet-dosing studies. Commonly cited studies involving waterfowl are inappropriate because they reflect exposure to particle lead that occurs through sifting ponded sediments with the bill, and because of the greater size of these birds relative to common upland grit ingesters (*e.g.*, quail).

Many of the studies cited are not ecologically relevant because the pellets used were considerably larger than those fired at trap and skeet ranges (*i.e.*, generally sizes 7.5, 8, or 9; Fimreite 1964; Gjerstad and Hanssen 1984), or because the dosing rates of the forced feeding experiments well exceeded what is reasonable for an upland bird to ingest (Buerger *et al.* 1986; Castrale and Oster 1993; McConnell 1968; Pattee *et al.* 2006). Still other studies are lacking in utility because they do not report on pellet retention times, manifested blood lead levels, standard blood parameters (*e.g.*, red blood cell count [RBC], blood packed cell volume), or altered delta aminolevulinic acid dehydratase (δ-ALAD) level, a prime indicator of lead poisoning (Paasivirta 2000). The subject study provides new information because it reports the aforementioned physiological measures in northern bobwhite (*Colinus virginianus*) and it does so for a longer time period (*i.e.*, 56 days post-dosing) than most pellet-dosing studies in the published literature. Further, spent shot pellets from a skeet range (what is termed here, "environmental shot"), as opposed to new (unfired) shot pellets, were used. In place of having the shiny grey-black metallic luster of unused pellets, spent shot pellets develop a crust of white, grey, or brown material (Jorgansen and Willems 1987) from the formation of lead oxides, carbonates, and other soluble lead compounds produced by weathering (Sever 1993). Other than a study by Kerr *et al.* (2010) and a 7-day study involving five birds of one sex by Vyas *et al.* (2001), spent shot has not been employed in any force-feeding pellet experiment. Heretofore, a few researchers incorporated pellet-weathering features in their experimental designs, but these efforts did not simulate the true ecological site condition (McConnell 1968; Rocke *et al.* 1997).

This study is a follow-up investigation of the Kerr *et al.* (2010) effort, a survivorship-oriented, range-finding, pellet-dosing study with the northern bobwhite. The Kerr *et al.* (2010) study implemented two high-dose lead pellet groups, and required euthanization of the groups early in the experiment; hence, dose–response curves for blood lead concentration and δ-ALAD activity could not be established. The study queried whether a single pellet dosed to the gizzard would be lethal. Such has become the operative question to answer in shooting range ERA investigations because of a popular model developed to assist with avian particle-ingestion assessments (Peddicord and LaKind 2000), and the rather commonplace misinterpretation of that model. The Peddicord and LaKind (2000) model estimates the probability of a bird ingesting a single spent shot pellet in its lifetime. Since the model's publication, ecological risk assessors have come to assume that because of the model's focus, death follows from the ingestion and retention of one shot pellet (USFWS 2002a,b). Importantly, all one-pellet dosed birds ($n = 10$) survived in the 28-day Kerr *et al.* (2010) study, suggesting that small upland birds can tolerate this dosage of incidental lead particle ingestion. In dosing northern bobwhites with up to three pellets at a time in the present study, exceeding what may be a realistic grit-ingestion rate (see Discussion), dose–response curves for blood lead concentration and δ-ALAD activity could be established. The fuller understanding of lead shot pellet toxicity presented in this article was realized by using the pellet retention time data from Kerr *et al.* (2010), obtained from radiography conducted in the same laboratory as part of a study that employed an identical experimental design.

STUDY DESIGN

Sixteen-week-old northern bobwhites were obtained from M & M Quail Farms, Gillsville, Georgia, an independent breeder. Following a 14-day quarantine period, 18 males and 20 females were selected at random and individually housed in quail cages fitted with an automatic watering system (Alternative Design, Siloam Springs, AR). Before the study began, birds were allowed 1 week of acclimation with *ad libitum* access to a pelleted sporting bird conditioner (Southern States, Richmond, VA), and 2 additional weeks with access to a seed-based diet (Pennington Pride; containing white millet, milo, wheat, sunflower seed, calcium carbonate, vitamin A and D3 supplement, potassium iodide, and vegetable oil) with fortified grit (Purgrain; high calcium with phosphorous, magnesium, potassium, and sulphur sources). Throughout the study, birds were on a 14:10-h light:dark cycle, room temperature of $23 \pm 5°C$, and humidity maintained at $45 \pm 10\%$. Body mass (as bodyweight) and feed consumption (as decreases in food volumes provided) were monitored weekly over the study period.

Spent shot pellets, identified as size 9, each weighing approximately 50 mg and assayed as being comprised of 90% lead (*i.e.*, 900,000 ppm) were obtained from an inactive skeet range at Joint Base Langley-Eustis (Fort Eustis), Virginia. One, two, or three pellets were administered to the gizzard by oral gavage ($n \geq$ four animals per treatment for both males and females) in 2-mL physiologic saline (Hospira, Lake Forest, IL) using a piece of rubber tubing attached to the end of a 3-mL dosing syringe. Control animals received physiological saline only.

All blood samples (0.5 mL) were collected from the jugular vein using aseptic technique. Samples were drawn with a 25-gauge needle on a 3-mL syringe at 0, 1, 2, 4, 6, and 8 weeks post-gavage. Samples were transferred to depressurized heparinized tubes, and mixed thoroughly before aliquots were removed for analyses. Blood lead levels were measured in duplicate 100-μL aliquots using the method described by Meldrum and Ko (2003). For whole blood cellularity, a 10-μL aliquot was transferred to a tube containing 990 μL of phosphate buffered saline (PBS) and gently mixed. A 10-μL sample of the mixture was added to a counting chamber containing 10-mL of PBS, and the cell suspension was enumerated with a Beckman Coulter Multisizer III (Beckman Coulter, Inc., Brea, CA). Packed cell volume (PCV) was determined by filling a capillary tube 3/4 full, stoppering the bottom of the tube by insertion into a clay pad, placing the tube into a spinner, centrifuging for 5 min, then determining the PCV with a graphic reader card. Capillary tubes were also used to determine total protein (g/100 mL). Tubes were cut above the RBC layer and approximately two drops of plasma were placed on a refractometer for measurement. The mean corpuscular volume (MCV) was determined (expressed in femtoliters; fL) by multiplying the PCV by 10, then the product was divided by RBC $\times$ $10^6/\mu$L. Delta-ALAD activity was determined by using the European standardized method (Berlin and Schaller 1974) and results were expressed as nanomoles of ALAD per minute per mL RBC.

After the 8-week blood samples were taken, each bird was euthanized with CO_2 gas as per institutional animal care and use committee approved procedures. The ventriculus, kidney, liver, spleen, sciatic nerve, and reproductive organs were collected, weighed separately, then each fixed in 10% buffered formalin before sectioning at 4–5 μm from samples embedded in paraffin wax. Sections on slides were stained with hematoxilyn and eosin, and examined by light microscopy. Sciatic nerve sections were also stained with Luxol Fast Blue to assess myelin degeneration. Slides were blindly scored from 0 (no degeneration) to 5 (severe degeneration) by a board-certified (American College of Veterinary Pathologists) pathologist.

Analysis of variance was conducted at each time point to test for differences among dose groups with regard to all measurements (*e.g.*, blood lead concentrations, δ-ALAD activity). Dunnett's *post hoc* tests were used to compare each group with the controls. Pearson's correlation was used to quantify the relationship between dose levels and blood lead concentrations. For all analyses, significance was determined at alpha = .05.

RESULTS

Appearance and Survivorship

At no time during the 56-day observation period did any pellet-dosed bird display any sign of illness. Qualitatively, none of the dosed birds appeared any less active relative to controls nor did there appear to be any compromised posturing. Three-pellet birds lost weight (although not a significant difference) but feed consumption did not decline. One male of a group of six one-pellet birds died during the study, possibly from overbleeding in one of the initial blood draws. Two two-pellet females died in the study, and all 11 of the three-pellet dosed birds (five males; six females) survived.

Blood Lead Levels

Sex-specific patterns of manifested blood lead levels and lead clearance rates were consistent across the three treatment groups (Table 1). In all cases, males achieved their maximum blood lead concentrations in Week 1, and steadily cleared the lead over the ensuing weeks. As part of their clearance profile, the transitions from Week 1 to Week 2, and from Week 2 to Week 4 were substantial. The one- and two-pellet males exhibited decreases of slightly more than 55% in the former, and greater than 82% in the latter. The clearance pattern for the three-pellet males was initially slower relative to the one- and two-pellet males; in the transition from Week 1 to Week 2, the blood lead reduction was approximately 28%. From Week 2 to Week 4, the percentage reduction (about 89%) mirrored the large-scale decreases of the one- and two-pellet male birds. Importantly, at 56 days post-dosing, all males had substantially cleared their lead. Although lead was present at multiples of the baseline level, all three dosing groups at 56 days post-dosing retained a highly consistent 2% of their maximum attained blood lead concentrations.

Females exhibited several trends with regard to their manifested blood lead levels and subsequent lead clearance that were different from that observed in males (Table 1). First, maximum attained blood lead concentrations were 23.2–28.2% lower than those observed in males. Variability associated with blood lead levels of females (SEM), was observed to be less than that of males. In what would otherwise be described as a stepwise blood lead clearance pattern with time, each female treatment group had at least one instance of an inversion (*i.e.*, a higher blood lead concentration occurring after a lower concentration had been achieved). It was further observed that females retained greater quantities of lead at study termination than males; whereas males retained 2% of their maximum attained concentrations, one-, two-, and three-pellet females, respectively, retained 30, 25.9, and 8.2% of their highest recorded lead concentrations.

Other Measured Blood Parameters

For two of the four measured standard blood parameters, MCV and PCV, there were limited cases of statistical difference between pellet-dosed birds and controls, with these differences occurring only in females (Tables 2 and 3). More specifically, the differences occurred in two- and three-pellet birds, and with one exception, only in weeks 6 and 8. For two other blood parameters, RBC counts (whole blood cellularity) and plasma total protein level (Tables 4 and 5), there were only single instances of statistical significance. These both occurred in males, one in Week 1 and the other in Week 8.

Histopathology

Analysis of the major organs revealed no abnormal clinical findings for pellet-dosed birds (*i.e.*, weight or histopathological abnormality relative to controls). In all treatment groups, there was some evidence of hepatic glycogen deposition and lipidosis. All birds had some level of pulmonary hemosiderosis, confirmed by Perl's iron staining. The cause of the pulmonary hemosiderosis could not be determined by histology. In females, a non-significant increasing trend in proximal tubular

Table 1. Peripheral blood lead levels (μL/dL)[1] in northern bobwhite[2] orally dosed with environmental lead shot pellets.

	Saline		One pellet		Two pellets		Three pellets	
Sampling event	Male	Female	Male	Female	Male	Female	Male	Female
Baseline	<1.2 ± 0.0	<1.2 ± 0.00	<1.2 ± 0.0	<1.2 ± 0.0	<1.2 ± 0.0	<1.2 ± 0.0	<1.2 ± 0.0	<1.2 ± 0.0
Week 1	<1.2 ± 0.0	2.6 ± 1.3	1297.5 ± 421.4	253.1 ± 14.3	1525.5 ± 485.9	417.9 ± 61.9	2781.5* ± 1120.6	645.4* ± 224.6
Week 2	<1.2 ± 0.0	3.6 ± 1.6	555.8 ± 226.4	365.9 ± 2.2	673.6 ± 306.5	135.0 ± 17.7	2000.0* ± 999.9	393.5* ± 173.0
Week 4	<1.2 ± 0.0	1.9 ± 0.6	55.2 ± 15.3	162.8* ± 35.3	118.2* ± 31.3	111.0 ± 42.8	223.7* ± 71.3	142.3* ± 35.0
Week 6	<1.2 ± 0.0	1.6 ± 25.2	41.6 ± 12.8	166.9* ± 9.8	70.5* ± 12.3	147.4* ± 24.7	70.5* ± 20.6	157.2* ± 13.7
Week 8	<1.2 ± 0.0	<1.2 ± 0.0	24.9 ± 4.4	111.6* ± 7.8	30.5* ± 3.8	108.1* ± 14.6	53.2* ± 13.8	52.7* ± 13.6

[1]All values are mean ± standard error of the mean (SEM); [2]n (nominally) = 5 per sex per treatment; $^*p \leq .05$ (Dunnett's test); asterisks highlight statistically significant differences between a test animal group and its control (physiological saline) counterpart (of the same sex and week). Because bleeding of quail by brachial or jugular vein is difficult, it was only attempted once/bird/week. As a result, group size fell to N = 2 for several exposure groups each week, precluding statistical analysis but showing consistent data trends. When the group size was $n \geq 3$, statistical significance was always achieved.

Table 2. Blood mean corpuscular volume (fL)[1] in northern bobwhite[2] orally dosed with environmental lead shot pellets.

	Saline		One pellet		Two pellets		Three pellets	
Sampling event	Male	Female	Male	Female	Male	Female	Male	Female
Baseline	17.5 ± 0.8	15.8 ± 1.3	18.3 ± 0.7	16.4 ± 1.1	17.8 ± 1.0	15.1 ± 0.9	16.7 ± 0.8	21.4 ± 6.7
Week 1	17.9 ± 0.6	15.0 ± 1.0	17.7 ± 1.2	17.4 ± 1.6	24.5 ± 4.5	18.2 ± 1.7	17.9 ± 0.7	19.4 ± 1.2
Week 2	16.5 ± 0.5	15.6 ± 1.2	15.6 ± 1.1	18.6 ± 1.1	17.1 ± 2.7	16.3 ± 0.8	15.6 ± 0.7	18.5 ± 1.6
Week 4	14.4 ± 0.6	14.2 ± 0.6	14.7 ± 0.8	15.0 ± 0.5	15.5 ± 0.8	15.0 ± 0.6	16.5 ± 1.3	14.9 ± 1.0
Week 6	16.6 ± 0.4	12.5 ± 0.7	15.1 ± 0.8	14.9 ± 0.7	15.4 ± 0.9	13.8 ± 0.7	16.0 ± 1.5	16.0* ± 1.3
Week 8	15.0 ± 0.8	9.5 ± 0.2	15.4 ± 0.7	12.5 ± 1.2	13.8 ± 0.3	14.7* ± 0.9	13.9 ± 0.4	14.4* ± 1.0

fL: femtoliters; [1]all values are mean ± SEM; [2]n (nominally) = 5 per sex per treatment $^*p \leq .05$ (Dunnett's test); asterisks highlight statistically significant differences between a test animal group and its control (saline) counterpart (of the same sex and week).

Table 3. Blood packed cell volume (%)[1] in northern bobwhite[2] orally dosed with environmental shot pellets.

	Saline		One pellet		Two pellets		Three pellets	
Sampling event	Male	Female	Male	Female	Male	Female	Male	Female
Baseline	48.2 ± 1.3	35.0 ± 2.9	46.4 ± 1.5	34.3 ± 1.0	48.8 ± 2.6	31.2 ± 3.5	47.0 ± 1.6	38.8 ± 1.1
Week 1	46.0 ± 1.2	33.8 ± 1.8	46.8 ± 2.1	34.0 ± 2.5	45.2 ± 1.2	37.6 ± 1.5	43.5 ± 2.1	38.0 ± 2.0
Week 2	44.6 ± 1.4	36.0 ± 1.2	45.2 ± 2.5	38.0 ± 1.0	43.8 ± 1.5	37.3 ± 0.9	43.2 ± 1.8	37.2 ± 1.7
Week 4	46.8 ± 1.4	34.5 ± 0.9	45.5 ± 1.2	35.6 ± 0.9	47.0 ± 1.1	36.3 ± 1.9	46.3 ± 1.7	39.2 ± 0.7
Week 6	47.3 ± 2.2	32.3 ± 0.7	48.4 ± 1.8	34.4 ± 1.1	46.8 ± 1.9	37.0* ± 0.6	48.0 ± 1.2	38.0* ± 1.6
Week 8	46.6 ± 1.7	31.0 ± 0.6	50.2 ± 2.1	34.4 ± 2.5	45.8 ± 1.7	36.0 ± 1.0	46.6 ± 2.1	37.2* ± 1.1

[1]All values are mean ± SEM; [2]n (nominally) = 5; $^*p \leq .05$ (Dunnett's test); asterisks highlight statistically significant differences between a test animal group and its control (saline) counterpart (of the same sex and week).

Table 4. Whole blood cellularity (red blood cell count; billions of cells/mL)[1] in northern bobwhite[2] orally dosed with environmental shot pellets.

	Saline		One pellet		Two pellets		Three pellets	
Sampling event	Male	Female	Male	Female	Male	Female	Male	Female
Baseline	2.8 ± 0.2	2.3 ± 0.1	2.5 ± 0.1	2.1 ± 0.1	2.8 ± 0.1	2.1 ± 0.3	2.8 ± 0.1	2.4 ± 0.4
Week 1	2.6 ± 0.1	2.1 ± 0.1	2.7 ± 0.1	2.0 ± 0.1	2.0* ± 0.3	2.2 ± 0.3	2.4 ± 0.0	2.0 ± 0.1
Week 2	2.7 ± 0.1	2.3 ± 0.1	2.9 ± 0.2	2.1 ± 0.2	2.7 ± 0.3	2.3 ± 0.1	2.8 ± 0.2	2.0 ± 0.1
Week 4	3.3 ± 0.2	2.5 ± 0.1	3.1 ± 0.1	2.4 ± 0.1	3.0 ± 0.1	2.4 ± 0.2	2.9 ± 0.2	2.7 ± 0.2
Week 6	2.9 ± 0.2	2.6 ± 0.2	3.2 ± 0.1	2.3 ± 0.2	3.1 ± 0.1	2.7 ± 0.2	3.1 ± 0.2	2.4 ± 0.2
Week 8	3.1 ± 0.2	3.3 ± 0.1	3.3 ± 0.2	2.9 ± 0.3	3.2 ± 0.2	2.5 ± 0.2	3.4 ± 0.1	2.7 ± 0.22

[1]All values are mean ± SEM; [2]n (nominally) = 5; *$p \leq .05$ (Dunnett's test); asterisks highlight statistically significant differences between a test animal group and its control (saline) counterpart (of the same sex and week).

Table 5. Plasma total protein levels (g/dL)[1] in northern bobwhite[2] orally dosed with environmental shot pellets.

	Saline		One pellet		Two pellets		Three pellets	
Sampling event	Male	Female	Male	Female	Male	Female	Male	Female
Baseline	4.7 ± 0.2	6.7 ± 0.6	5.1 ± 0.2	7.1 ± 1.4	5.3 ± 0.3	7.2 ± 1.1	5.5 ± 0.2	7.4 ± 0.6
Week 1	4.9 ± 0.3	5.5 ± 0.3	5.2 ± 0.4	5.4 ± 0.7	5.3 ± 0.3	6.2 ± 0.6	6.0 ± 0.4	6.1 ± 0.4
Week 2	5.1 ± 0.4	6.1 ± 0.6	5.4 ± 0.5	6.4 ± 0.9	5.8 ± 0.5	5.6 ± 0.4	5.6 ± 0.5	5.9 ± 0.7
Week 4	4.7 ± 0.2	5.8 ± 0.2	5.4 ± 0.4	6.4 ± 1.1	4.9 ± 0.2	8.0 ± 2.0	5.8 ± 0.5	6.6 ± 0.6
Week 6	4.2 ± 0.9	4.9 ± 0.4	5.1 ± 0.4	6.0 ± 0.3	5.1 ± 0.2	5.9 ± 0.2	5.8 ± 0.5	6.0 ± 0.4
Week 8	4.7 ± 0.4	5.5 ± 0.8	5.3 ± 0.4	6.4 ± 0.7	5.0 ± 0.2	5.8 ± 0.2	5.9* ± 0.5	6.6 ± 0.7

[1]All values are mean ± SEM; [2]n (nominally) = 5; *$p \leq .05$ (Dunnett's test); asterisks highlight statistically significant differences between a test animal group and its control (saline) counterpart (of the same sex and week).

degeneration in kidneys was also observed. No myelin degeneration was observed in the sciatic nerve sections.

δ-ALAD Activity

There was considerable suppression of δ-ALAD activity over the 8-week time period (Figure 1). Beginning with Week 1, all dosing groups exhibited reductions in δ-ALAD activity relative to baseline. For both sexes and all three pellet-dosing groups, the greatest degree of enzyme suppression occurred at Weeks 1 and 2. Some recovery toward baseline activity was evident in all birds beginning at Week 4. Whereas males showed a steady improvement over the last three sampling events, with one-pellet birds notably at full recovery (*i.e.*, at the level of baseline) by Week 8, each of the female treatments displayed a different trend. The severely suppressed activity in one-pellet females only minimally varied over the 8 weeks. After an initial recovery, two-pellet birds showed a steady decline in Weeks 4 through 8. Curiously, three-pellet birds showed increases before leveling off.

DISCUSSION

Health/Survivorship

Our data suggest northern bobwhite that are pellet-dosed in an ecologically relevant manner (*i.e.*, with environmental shot as opposed to unused shot, and force-fed one-three shot pellets) can tolerate lead exposure. For both males and females, this determination is first supported by the survivorship statistics and the absence of any signs of illness over the 56-day observation period. It is critical to note, however, that the meaningfulness of this study's two- and three-pellet exposures is veiled in uncertainty. The Peddicord and LaKind model and others similar to it (Luttik and deSnoo 2004) assume, in part, that pellet ingestion behavior is isomorphic to grit ingestion behavior, and this may not be true. Although upland birds have been observed in the wild with multiple grit particles in the gizzard, documentation of birds ingesting more than one grit particle on the same day does not exist. It is acknowledged that such might be due to limited study of the phenomenon. Similarly, although upland birds in the wild have been found with multiple pellets in the gizzard (Hunter and Rosen 1965; Lewis and Schweitzer 2000), there is no documentation of birds ingesting more than one shot pellet at one time (*i.e.*, on the same day). To the extent that the study's simultaneous two- and three-pellet exposures are forced and possibly unrealistic, the high survivorship associated with these exposures (including 100% survival for all three-pellet males and females through 56 days) supplies a layer of confidence when concluding that bobwhite exhibit high tolerance to the particle lead exposures.

Because no deaths occurred among two- and three-pellet males, it is likely that the singular death in the one-pellet group is an outlier, and possibly attributable to a hematoma following blood collection. This possibility is supported by the scheduled weekly and biweekly 0.5-mL blood draws meeting safe blood collection recommendations (Fair *et al.* 2010). The singular male loss of this study should be reviewed in the context of survivorship statistics of the Kerr *et al.* (2010) study in which no

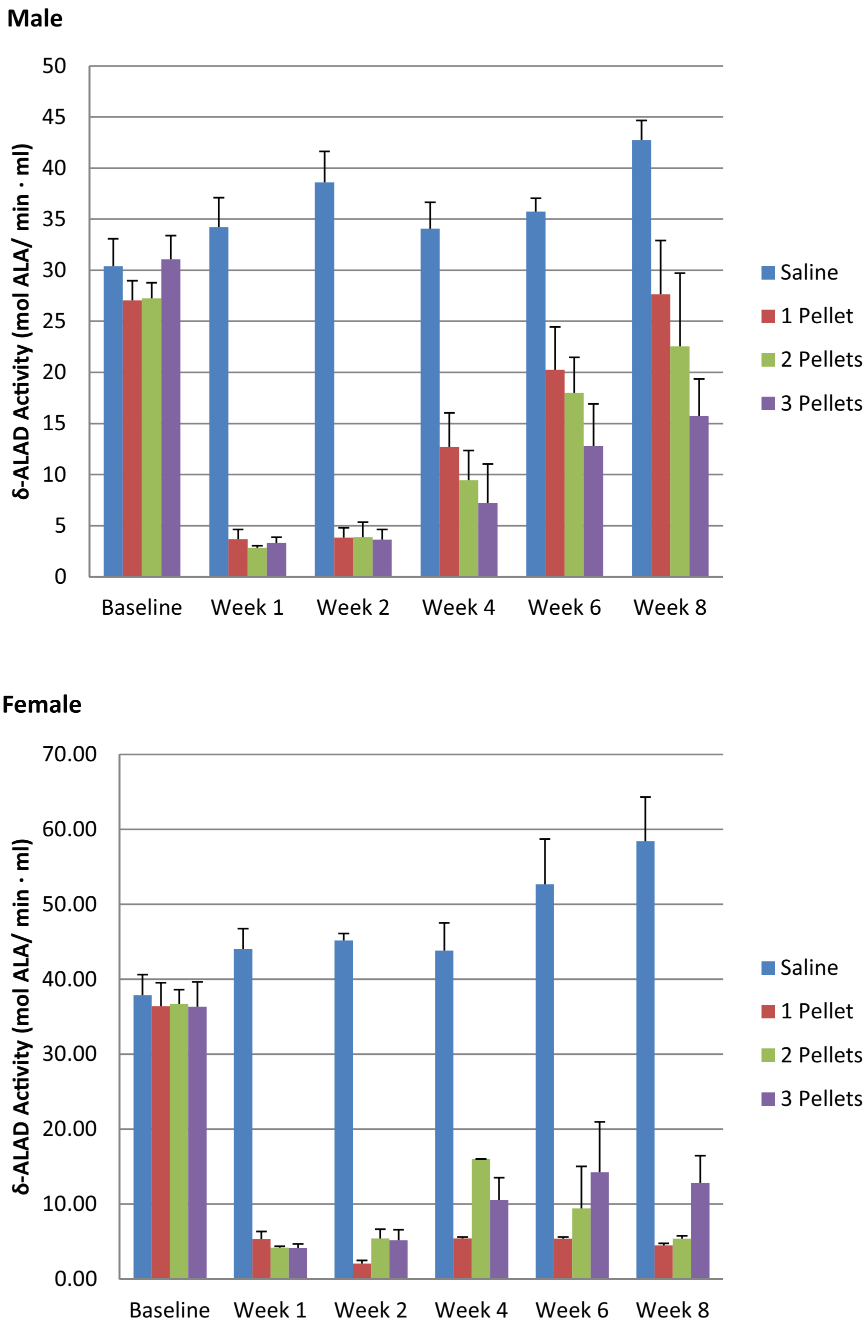

Figure 1. δ-ALAD activity in male (top) and female (bottom) northern bobwhite over the 56-day monitoring period following environmental lead shot gavage. All dosed birds had lower δ-ALAD activity than controls within each time period ($p \leq .05$, Dunnet's test). (Color figure available online.)

birds died. The Kerr *et al.* (2010) study was conducted about 6 months before this study, under the same experimental conditions within the same laboratory. There were no deaths in a group of five, one-pellet exposed males of the Kerr *et al.* (2010) study. Thus the one lost bird of the subject study relates to a universe of 11, and thereby a survivorship rate of 91%. Although the subject study's dosings of two and three pellets at one time are not particularly realistic, there is value in knowing that all males survived them. It is suggested that the appropriate universe to consider in computing the male survivorship rate includes the 10 birds that received two or three pellets at a time. Survivorship among this larger group of 21 males was slightly more than 95%. Similar survivorship computations are applicable to the females of the subject study. All females dosed with one pellet survived. If data from one-pellet females are pooled with two- and three-pellet females, and with the one-pellet females of the Kerr *et al.* (2010) study, survivorship would be 90%. It is acknowledged that survivorship in the wild might be lower owing to birds needing to expend greater energy in coping with various environmental conditions.

Histopathology

The hepatic glyocogen deposition and lipidosis observed in all treatment groups is likely attributed to the seed diet, although there is no information to support this. It is likely that the proximal tubular degeneration observed in the female kidneys can be attributed to the dehydration that accompanies lead treatments.

Blood Lead Levels and δ-ALAD Activity

The inability of up to three force-fed environmental shot to compromise health is further demonstrated by the bobwhite's tolerance of pronounced blood lead levels. This was particularly evident in the maximum levels assumed, with males exceeding the reported severe clinical poisoning threshold (100 μg/dL; Franson and Pain 2011) by more than 25-fold, and females by more than 6-fold. Despite the demonstrated blood lead tolerances, the dissimilar blood lead clearance patterns of males and females (*i.e.*, males with a stepwise decrease throughout post-dosing, and females with sporadic instances of higher blood lead concentrations arising after concentrations had been decreasing) are deserving of attention. It is believed the difference can be traced to ovulation during the study. The observed instances of blood lead level inversion suggest that females were sequestering lead in various compartments (*e.g.*, bone) while in production, and secondarily releasing the lead from the compartments when it was opportune to do so.

Recalling that the only truly ecologically relevant dosing of the subject study was that of the one-pellet birds, the recovery to baseline of the initially suppressed δ-ALAD activity in one-pellet males suggests that these animals' health was not challenged. Their return to baseline δ-ALAD activity at 56 days notably parallels the clearance of 98% of the blood lead at that time point, and logically the voided lead is the cause of enzyme activity no longer being suppressed. Explaining δ-ALAD activity recovery or the lack of it in females is seemingly straightforward. Beginning with Week 4, one-pellet females in almost every instance, had higher blood lead levels than did two- and three-pellet females, and presumably again, manifestations of these birds ovulating accounts for this finding. It is not clear though, why three-pellet females that initially attained the highest blood lead levels of any of their female counterparts, cleared more lead from their blood by Week 8 than their one- and two-pellet counterparts. Why three-pellet females at 8 weeks had better recovery of their δ-ALAD activity (albeit poor, nevertheless) than did all other females, follows again from their relatively lower blood lead levels in the later weeks of the study.

Are female birds with sustained suppression of δ-ALAD activity unhealthy? In mammals, δ-ALAD activity is tied to heme synthesis, and depressed δ-ALAD levels can be associated with an inability of blood cells to transport oxygen. In mammals also, nausea, vomiting, and behavioral changes are consistent with δ-ALAD activity-caused impairment of RBC function. Conceivably birds should be similarly affected, although the subject study recorded no such signs of illness, even when males and females were experiencing their greatest degrees of enzyme suppression. It may be inappropriate to extrapolate health effects experienced in enzyme-suppressed mammals to health effects in birds, because mammalian RBCs are anucleated, whereas avian RBCs are nucleated. With birds also having a relatively higher RBC regenerative capacity (Feldman *et al.* 2000), they may have a higher tolerance to lead. Potentially, the absence of any observed health effects in any of the subject study's female birds (that presented with delayed lead clearance and extended δ-ALAD activity suppression) is due to their RBC design and their overall tolerance to lead.

Are female birds with blood lead burdens and sustained δ-ALAD activity suppression at risk, and in particular, where it is also unclear how much more time is needed until recovery? More broadly, are birds that demonstrate a capability to fully recover from such insults, such as the males of the subject study, at risk until such time as they achieve full recovery (evident in all blood lead having been cleared)? These questions are effectively probing the consequences of a bird in the wild ingesting a spent shot pellet before fully recovering from the effects of having ingested a first one. To answer the questions, it is important to realize that manifested blood lead levels and measured δ-ALAD activity cannot be reviewed in a vacuum. These phenomena necessitate considerations of pellet retention times, the nuances of both grit and spent shot ingestion behavior, and an awareness that inside the body, shot pellets may not behave like actual grit particles. Regarding the first of these, having the shot pellet retention times of the earlier study is most fortunate, for these are the only existing data of that kind. As for grit retention times, there are few studies that directly measure mean or median values (USEPA 2011). For grit ingestion behavior, which can undoubtedly influence pellet ingestion behavior, only general trends are known. As examples, larger birds tend to select larger grit particles, species-specific grit preferences on the basis of color, shape, and texture are acknowledged to exist, and grit uptake is governed to varying degrees by its availability (Best and Gionfriddo 1991; Gionfriddo and Best 1996, 1999).

In all birds of the Kerr *et al.* (2010) study, pellet retention was at 21% in the first week after dosing, at 7% after 2 weeks, and at 0% by the 3rd study week. These retention times are notably shorter than what is reported in the literature for true grit. Additionally, the transit rate of environmental shot through the GI tract was observed to be independent of the number of gavaged shot, and there appeared to be no correlation of the number of gavaged and the number of retained shot. The retention times also corroborate the recent findings for another lead form similarly dosed to the gizzard in bobwhite, namely bullet fragments (Kerr *et al.* 2011). This study reported retention times for bullet fragments of no more than 1 week. Relatively brief pellet retention times convey several noteworthy pieces of information. First, the accelerated pace of the excretion of gavaged pellets limits the opportunities for pellets to entirely dissolve inside the bird. With pellets not fully dissolving before excretion, birds are spared achieving what would undoubtedly be blood lead levels still higher than those observed here. Alternatively, short pellet retention times could mean that birds are being readied that much more often to seek out a new grit or grit-like particle, which in theory could again be a shot pellet. This assumes that grit-ingesting birds have a feedback mechanism in place for sensing (with the aid of either sphincter control or tactile receptors in the proventriculus mucosae; Mathiasson 1972; McCann 1939) when they have excreted a grit particle, and also, whether or not the gizzard is stocked with a species-appropriate "fixed load" of grit particles (USEPA 2011).

Other Measured Blood Parameters

The finding of almost no cases of statistical difference between pellet-dosed and control bobwhites for MCV, PCV, whole blood cellularity, and plasma total protein levels corroborates the absence of observed clinical effects, and the demonstrated tolerance of high blood lead levels. Conceivably, had there been greater numbers of birds per treatment, more cases of significance might have occurred. At the same time, it is recognized that the subject study's small sample size and high variability could be masking differences that would otherwise be evident. Where there would be additional cases of significance, these would first be anticipated for the two- and three-pellet birds, since the cases of significance that occurred with our testing pertained to these, and also because these birds manifested the higher blood lead levels.

Ecological Considerations

For a bird that has ingested an environmental shot pellet, the probability that a next ingestion event will also involve an environmental shot pellet is probably quite limited when consideration is first given to relevant spatial and temporal dynamics. In many cases, shot fall zones of trap and skeet ranges are only 2 or 4 ha in size (National Shooting Sports Foundation 1997). Realistically a bird's foraging area encompasses more land than this, and consequently, the chance that a bird's next grit ingestion event will occur at the same fall zone is reduced. Urban (1972), for example, found a mean home range size of 16.7 ha for the northern bobwhite. Further, densities of grit ingesters are unlikely to be sufficiently high such that population level impacts, in a regional sense, would bear out. In the case of bobwhite, so relevant to the subject study, the literature reports a density of five birds/ha (or about two birds/acre; USEPA 1993). Temporal considerations include grit ingestion events being separated by perhaps weeks at a time, and birds being present at a shooting range for only a subset of days (the latter a consideration of the Peddicord and LaKind model). Taken together, the probability of a bird returning to the same location for its next corrupted grit ingestion event (*i.e.*, an environmental shot pellet mistakenly taken as grit) while still retaining excess blood lead is again seen to be unlikely.

Still other considerations reduce the likelihood of consecutive spent shot pellet ingestion events. Although the subject study facilitated spent shot pellet ingestion and gizzard retention through a force-feeding design, grit-ingesting birds might not prefer the oral sensation of spent shot pellets altogether. Unlike true grit, spent shot pellets are spherical, have a metallic taste, and are considerably denser than equivalently-sized bits of rock or stone. Since spent shot pellets have features that so radically depart from true grit, various species of birds in the wild might conceivably reject shot pellets only shortly after being taken into the mouth. It is important to note that this study's facilitation of pellet ingestion and retention nevertheless maximized opportunities for pellets to erode and to allow for lead accumulation in the bloodstream. A study with sparrows and red-wing blackbirds (*Agelaius phoeniceus*) found that soft bird diets (consisting of insects, generally) do not require much grit, if any, to aid digestion (Fischer and Best 1995). Further, grit particles ingested by insectivorous birds tend to be relatively rapidly eliminated (USEPA 2011). Our deliberate choice then, of a hard (*i.e.*, seed-based) diet was intended to maximize lead exposures by extending pellet retention times somewhat, as is theorized to occur (USEPA 2011), and to foster a relatively faster rate of lead particle erosion (Chasko *et al.* 1984). At the same time it is recognized that the seed-based diet containing high calcium could have had the effect of inhibiting the dietary absorption of lead (Carlson and Nielse 1985; Scheuhammer 1996).

It is noteworthy that most dosed pellets in the supporting Kerr *et al.* (2010) study were excreted within a week, and that none were retained beyond 3 weeks. The assumption then, that ingested shot pellets behave quite similarly to ingested grit particles, may not be a fair one in light of literature reporting northern bobwhites retaining grit particles from as little as 6 weeks to as long as 9 months (Errington 1931; Nestler 1946; Robert and Bisset 1979). In accounting for this difference, it is suggested that the spherical pellets might not securely lodge against the inner lining of the gizzard as well as do true grit particles. The suggested phenomenon lends itself to study.

Two final ecological considerations germane to the topic of incidental lead particle ingestion by upland birds bear on the potential for toxic exposures to arise, and for lead pellet ingestion to constitute a legitimate ERA concern overall. First, upland birds are never described in the literature as absolutely requiring grit. This is supported by preference studies where birds did not ingest any grit set out before them if the choices did not include certain species-specific characteristics for such factors as color, size, and angularity (Best and Gionfriddo 1991). Birds sooner forego ingesting spent shot pellets, even where they are plentiful, when pellet features do not match those of the grit type the birds ordinarily consume. The second consideration is one of the anticipated numbers of birds of any given species to occupy the fall zones of trap or skeet ranges. It is acknowledged though, that few population-level studies have proceeded at lead shot fall zones. Conceivably though, numbers of birds are relatively low within these 2 to 4 ha areas, and seemingly this explains the absence of accounts of population-level impacts at such locales. A case in point is that of the northern bobwhite with a reported highest density of five birds per ha (USEPA 1993). When consideration is given to population size being highly subject to seasonal variation, maximum reported densities are likely to be misleading, and there are significantly reduced opportunities for sizeable numbers of birds of a given species to entertain pellet ingestion behavior.

CONCLUSIONS

Despite a sustained suppression of δ-ALAD activity, particularly in females, northern bobwhites appear to be sufficiently resilient and do not succumb to poisoning associated with ecologically-relevant doses of lead shot. For both sexes, survivorship over an observation window allowing for substantial, if not total, blood lead clearance was $\geq$95%. Birds that received as many as three pellets at one time, a dosing rate not described in the open literature and not necessarily anticipated to occur in the wild, did not exhibit any overt signs of illness. Additionally, neither standard blood parameters nor major organ histology was compromised. Bobwhites in this study weighed about 125 g and tolerated force-fed lead exposures well; conceivably other grit-ingesters of equivalent size are similarly tolerant of ecologically-relevant shot pellet ingestion exposures. Reasonably, larger grit-ingesting species should also be able to withstand incidental lead pellet ingestion and there is recent evidence to bear this out (Gogal *et al.* 2012).

Given the demonstrated high survivorship of bobwhite in this study, published clinical lead poisoning thresholds for birds are presumably somewhat erroneous. Seemingly the thresholds are not altogether applicable in the assessment of lead ingestion exposures for many common upland grit-ingesters because the thresholds refer to Anseriformes and Falconiformes, species with different behaviors and ecology. The apparent imprecision of the thresholds likely stems from the use of models that endeavor to forecast blood levels using soil or dietary lead concentrations.

This study's findings suggest that the ERA concern of upland bird losses occurring at trap and skeet ranges may be unwarranted. The ERA community has used the Peddord and LaKind model to determine that single-pellet exposures are lethal to birds because the model calculates the probability of a bird ingesting one pellet in its lifetime. The model, however, was designed to generate such a probability only to supply exposure assessment information, and importantly, the model does not facilitate risk calculations. Additionally, the model does not allow for the calculation of safe pellet densities on the ground because an unhealthful condition would first need to be demonstrated and the means for doing so do not exist. Until the present study and its forerunner (Kerr *et al.* 2010), the toxicological effects of singular pellet exposures were essentially unknown, and any earlier attempts to understand the phenomenon were not ecologically relevant because they used new pellets, not those exposed to environmental conditions. It appears that appropriate dosing studies (*i.e.*, those employing environmental shot and administering only minimal numbers of these) support the finding that adverse effects of lead shot on bird populations are less of a concern than previously thought.

With pellet retention times observed to be particularly brief (about 2 weeks), pellets do not fully dissolve by the time they are excreted. The brief retention times mean lesser lead accumulations in the bloodstream but possibly too, shorter times until a bird might mistakenly ingest another spent shot pellet. Three areas of further investigation would substantially improve our understanding of lead pellet ingestion in upland birds as it may occur at trap and skeet ranges: establishing the potential for reproductive impacts to become manifest in δ-ALAD activity-suppressed birds, establishing the health consequences of birds being dosed with environmental shot anew before fully recovering from first pellet exposures, and conducting shot pellet-versus-grit preference studies.

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