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Mortality and Hematology Associated with the Ingestion of One Number Four Lead Shot in Black Ducks, *Anas rubripes*

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The black duck (*Anas rubripes*) is a dabbling duck endemic to North America. Since population surveys of this species began in the early 1950's, numbers have declined steadily at the rate of approximately 1.5% a year (Smith 1983). In 1980 the North American population was estimated to be only 42% of that recorded in 1952. Several reasons have been suggested for this decline. These include excessive hunting pressure, habitat loss, interspecific competition and hybridization with mallards (*Anas platyrhynchos*) and environmental pollution. Habitat loss has been reduced by wetland protection legislation introduced in the 1970's, but still occurs on a large scale. Special black duck hunting regulations were introduced in 1961 and strict bag limits exist. So far these measures have not checked the population decline.

One environmental contaminant that may be contributing to the decline is spent lead gunshot. The ingestion of gunshot by waterfowl is well documented (Sanderson and Bellrose 1986) and is thought to kill an estimated 1.6-3.8 million waterfowl each year in North America (Feierabend 1983). In the 1950's a nationwide survey of the incidence of shot ingestion by waterfowl was conducted (Bellrose 1959). The incidence of at least one ingested shot being found in the gizzards of dabbling ducks killed by hunters was recorded as 5-10%. There has been little research concerning the toxicity of lead gunshot to the black duck and the present significance of lead poisoning as a mortality factor in this species.

This study reports the results of an experiment in which captive black ducks were dosed with one number four lead shot. Mortality rates and hematological effects are discussed in relation to lead toxicity.

MATERIALS AND METHODS.

In August 1985, sixteen adult black ducks (8M, 8F) were randomly selected from a pure bred colony at the Patuxent Wildlife Research Center. Birds were housed individually in partially shaded outdoor wire mesh cages, ($1m^2$), raised above a concrete slab. Ducks were provided with water and Duck Breeder Beacon pellets (The Beacon Milling Co., Cayuga N.Y.).

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This diet is considered to be nutritionally balanced and high in calcium (33,200 ppm). Birds were dosed one day after being moved to cages. On the first day of the study (day 0), ten birds (5M, 5F) were orally dosed with one No. 4 lead shot from a Federal shotgun shell. Shot were administered via a flexible plastic tube inserted to the level of the proventriculus. Control birds (N=3M, 3F) were introduced at day three of the study, and were sham-dosed (i.e., intubated without shot administration). As this experiment was not originally designed to investigate the mortality of black duck after shot ingestion, no controls were originally called for. However, when unexpected mortality was observed, control birds were added (on day 3). Prior to dosage or sham-dosage all birds were weighed and blood samples were collected by jugular venipuncture. Dosed birds were weighed and bled at 1, 3, 6, 9 & 33 days post-dosage. Control birds were weighed and venisected at 1, 3, 6 & 30 days post sham-dosage. A similar volume of blood was taken at each sampling stage from dosed and control birds. This was 4ml at day 1, 3ml at day 2, and 2-2.5ml on each subsequent occasion. Blood was taken directly into potassium/heparin syringes to prevent sample coagulation. Samples were immediately chilled on ice.

Red blood cell δ-aminolevulinic acid dehydratase (ALAD) was determined within four hours of sampling [optimised for black ducks with pH 6.4 buffer, adapted from the method of Berlin and Schaller (1974)]. Hematocrits were determined on fresh blood and hemoglobin concentrations determined using the cyanmethemoglobin method (on centrifuged hemolysate, to remove nuclear material). An aliquot of each sample was stored at $4^{o}C$ for 48 hours prior to determination of zinc protoporphyrin (ZPP) (Roscoe et al. 1979). Approximately 1ml of whole blood was frozen at $-70^{o}C$ for subsequent blood lead analysis by electrothermal atomic absorption spectrophotometry (*Pain, in prep.*). Birds suffering mortality were necropsied.

Non-parametric statistics (Mann Whitney Tests) were used to compare median levels of the parameters measured in control and dosed birds since distributions were not normal. Results were considered significant when $p<0.05$.

RESULTS AND DISCUSSION.

Sixty percent mortality was recorded in black ducks which were provided a nutritionally balanced diet when dosed with one number four lead shot. One bird died on day four post-dosage, three birds on day five and two on day six. Of the birds that succumbed, four were female and two were male. All birds that died exhibited signs of acute lead poisoning by day 3 post-dosage. These typically included green watery faeces, muscular paralysis (drooping of tail and wings) and lethargy. Two of the birds that survived exhibited these signs in full and two exhibited only lethargy. The concrete below the cages was checked for the presence of excreted shot but none was observed. At necropsy all six birds were found to have retained the shot, which was only slightly eroded. All birds had pale (anemic) viscera, heart, lungs and bone marrow, and bile-stained livers. All birds were in good flesh. There was no mortality in control birds.

FIGURE 1

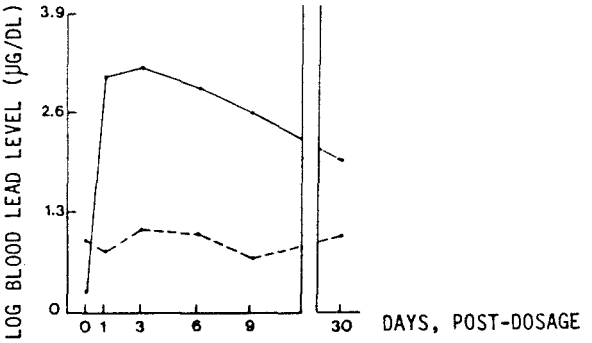

Figure 1 illustrates temporal changes in median blood lead (PbB) levels of black ducks dosed with one number 4 lead shot. Pre-dosage PbB levels were similar in dosed, ———(5M, 5F), and sham-dosed, - - - - - (3M, 3F) birds. From one day to one month post-dosage PbB levels were significantly higher in dosed birds (Mann-Whitney, $p<0.03$). PbB levels increased at a similar rate in all dosed birds.

The mortality recorded in dosed birds is unprecedented. Similar experiments in which pen-reared mallards were fed a nutritionally balanced diet and dosed with one No.4 lead shot resulted in little or no mortality (Finley et al. 1976, *Srebocan and Rattner, in prep.*). However, when pen-reared mallards, similarly dosed, were fed a nutritionally imbalanced diet, up to 20% mortality was recorded (Longcore et al. 1974a,b).

Weight loss of both control and dosed birds was fairly rapid. Dosed birds did not lose significantly more weight than controls. Body weights of both control and dosed birds were significantly lower (by 20%) than initial weights at six days post sham or lead dosage ($p=0.008$, 0.001 respectively). By one month post dosage body weights of all surviving birds were back to the initial level. The weight losses recorded here would probably have been lower had the birds been allowed additional time to become acclimatized to the cages.

Blood lead levels in control birds remained fairly constant throughout the experiment. Post dosage, blood lead levels were consistently greater ($p<0.03$) in dosed birds than in controls (Figure 1). In dosed birds the median blood lead level increased from 2 µg/dl at day 0 to 1,214 µg/dl within 24 hours. This increase implies very rapid absorption of lead into the bloodstream.
Hematocrit and hemoglobin concentrations remained constant for control birds throughout the experiment. Levels of both began to drop at day three post-dosage in dosed birds (Figure 2), and were significantly lower ($p<0.025$) than controls by day six. In the four surviving dosed birds levels of both parameters began to increase after day six and initial levels were re-established by one month. It is possible that the shot were expelled by the four surviving birds, but not detected.

ZPP and ALAD are two heme-biosynthetic parameters that are widely used to diagnose lead poisoning in waterfowl (Sanderson and Bellrose 1986). Levels of both remained relatively constant in controls for the duration of the experiment (Figure 3). Concentration of ZPP increased slightly at day 1, and greatly between days 3 & 6. Levels appeared to decline after day 9 post dosage, but remained significantly higher

FIGURE 2

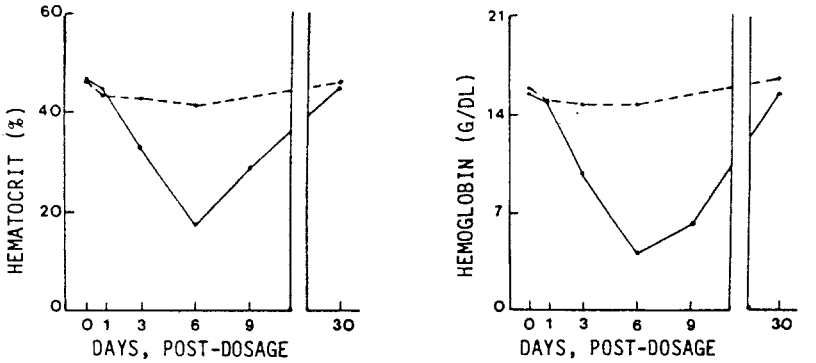

Figure 2 illustrates temporal changes in (a), hematocrit, and (b), hemoglobin levels of black ducks dosed with one number 4 lead shot. Hematocrit values were significantly lower in dosed birds, ————, than control birds,- - - - - -, at day 6 (Mann Whitney, p=0.023). Hemoglobin values were significantly lower in dosed birds at days 3 and 6 (Mann Whitney, p=0.008). Both hematocrit and hemoglobin levels were slightly lower (p=0.07) in dosed birds that died than those that survived at day three.

FIGURE 3

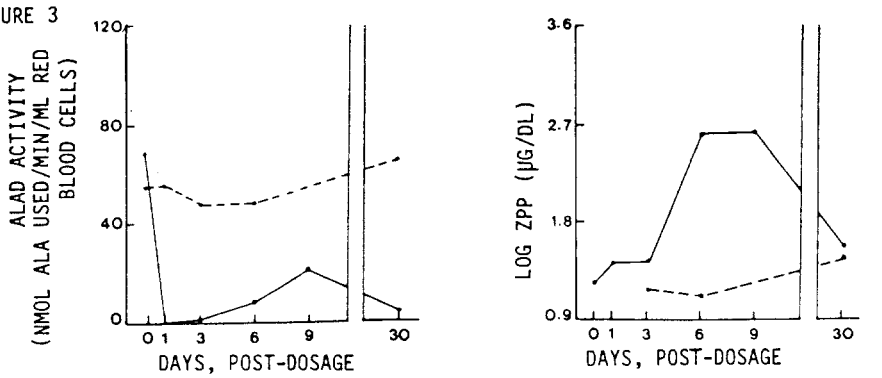

Figure 3 illustrates temporal changes in ALAD activities and ZPP concentrations of black ducks dosed with one number 4 lead shot. ALAD activities, (a), were significantly lower in dosed birds, ————, than control birds,- - - - - -, from one day to one month post-dosage (Mann-Whitney, p=0.000-0.002). ZPP concentrations, (b), were significantly higher in dosed birds from day 3 to one month post-dosage (Mann-Whitney, p=0.008-0.050). ALAD activities decreased and ZPP concentrations increased at a similar rate in all dosed birds.

($p<0.05$) than controls throughout the experiment. ALAD activities in all dosed birds were inhibited by 100% at day one post-dosage. This degree of inhibition is greater than that previously recorded (Finley et al. 1976; Dieter and Finley 1979; *Srebocan and Rattner, in prep*). ALAD activities were significantly lower than controls throughout the experiment (p=0.000-0.035). ALAD activities increased in dosed birds between three and nine days post dosage before falling back to a lower level of activity at one month post-dosage. This increase suggests that extra ALAD may be produced as a result of lead intoxication. This is supported by the results of experiments in which lead inhibited ALAD has been reactivated post dosage and been found to be far greater than initial levels, both in birds and other species (Kajimoto et al. 1983; *Pain, in prep.*).

The level of mortality recorded in this experiment is far higher than has been recorded for other species with similar treatments (Finley et al. 1976, Longcore et al. 1974a,b). The rapid increase in blood lead, and corresponding total inhibition of ALAD 24 hours after ingestion of just a single shot is also unprecedented. The extreme physiological responses observed may be partly due to the lack of a period of acclimitization to the cages. Although dosed birds lost weight at a similar rate to controls, the consequent nutritional deficit may have enhanced the toxicity of the lead. It is widely acknowledged that nutritional deficiencies, especially calcium deficiency, can enhance the toxicity of lead. Mallards dosed with 1-6 lead shot and fed a low calcium diet have shown a more than ten fold increase in tissue lead concentration over those fed a high calcium diet (Koranda et al. 1979). In addition to nutritional deficiencies, the extremely high ambient temperatures (max. 37.6^{o}C) that prevailed for the duration of the experiment may have influenced the lead toxicity. When ambient temperatures reach a certain critical level a bird's metabolic rate will increase, in turn producing more heat (Lewies 1969; Lack and Campbell 1985). Above this level heat is lost evaporatively (Dawson and Tordoff 1964), and water uptake may increase as a result. It seems reasonable to suggest that the rapid absorption of lead and associated mortality may have resulted from a temperature induced increase in metabolic rate. Investigations have shown lead toxicity to be increased with high ambient temperatures in other species (Edwards and Beatson 1984; Horiguchi et al. 1979). However, this hypothesis conflicts with the results of recent work investigating heat exposure and lead toxicity in mallards (*Srebocan and Rattner, in prep.*). No mortality was observed in mallards dosed with one No. 4 shot and maintained at either 21^{o}C or 35^{o}C in environmental chambers.

The apparent sensitivity of the black ducks in this experiment to lead shot is unexplained. A similar experiment in which mallards and pen-reared black duck were dosed with one number four lead shot showed no mortality (*Rattner and Fleming unpub. data*). It is possible that under certain undefined stress conditions lead toxicity may be more serious for black duck, and other waterfowl, than has been previously realized. Further investigations are required before questions can be answered relating the toxicity of lead gunshot to black duck and its potential contribution to the population decline.

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