Effects of Diet and Soil Ingestion on the Toxicity of Zinc Shot to Game-Farm Mallards

Final Report

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SUMMARY

We conducted a 30-day Acute Toxicity Test of Zinc (Zn) shot using 120 female, 6 to 8 month-old, wild-type game-farm mallards (*Anas platyrhynchos*). Sixty ducks were dosed with 8 No. 4 shot pellets containing 98% Zn and 2% tin (Sn), and the remaining 60 ducks were sham-dosed and served as controls. Fifteen ducks from each of the 2 dosing groups were assigned to one of 4 diet treatments: corn only (CORN); corn with soil available (CORN/SOIL); commercial duck pellets only (PELLETS); commercial duck pellets with soil available (PELLETS/SOIL).

The Zn shot dose resulted in high mortality (48.3%). Mortality rates were highest in the CORN and PELLETS/SOIL groups, however, survival rates did not differ significantly among dietary treatments. Survival in Zn-dosed ducks averaged 22.7 days. Although ducks in the CORN/SOIL and PELLETS groups survived longer, on average, than those in the PELLETS/SOIL and CORN cohorts, these differences were not significant.

A large number of Zn-dosed ducks displayed ataxia/paresis (68.3%). Although the number of ducks exhibiting these signs did not differ significantly among treatments, fewer ducks exhibited signs when soil was available, both within and between diets (corn and pellets).

Body weight averaged less in Zn- than in sham-dosed ducks at Days 15 and 30. The mean weight of Zn-dosed ducks decreased between Days 0 and 15, and increased from Day 15 to Day 30 (includes those that died during that period). Zn-dosed ducks which succumbed to Zn toxicosis lost a considerable proportion of their body weight between Day 0 and death. Mean body weight at Day 30 was greater in ducks fed commercial ducks pellets than those provided
corn and soil.

The shot retention rate was 63.6%, and the mean number of shot retained/duck was 5.8 pellets. Shot retention did not vary by diet. Shot dissolution rates ranged from 0.016 g/day in Zn-dosed ducks that retained 8 shot and died prior to Day 30, to 0.025 g/day in those that survived to Day 30. The gizzards of sham-dosed ducks contained more grit when compared to all Zn-dosed ducks, however, this difference was not apparent when the Zn-dosed cohort which died prior to Day 30 were excluded. Upon dissection, the gizzards of ducks provided soil contained a greater amount of grit than those of ducks not given soil.

The livers, pancreases, gonads, and gizzards were reduced, and kidneys enlarged, in Zn-dosed ducks relative to sham-dosed controls. The pancreas and gonad represented a greater proportion of body weight in sham-dosed ducks, while the kidneys accounted for a greater percentage of total body weight in Zn-dosed mallards. The livers, pancreases, gizzards, and gonads were lighter, and the kidneys were heavier, in ducks that died as a result of Zn intoxication than in Zn-dosed ducks which survived 30 days. In addition, the kidneys increased, and gizzards and gonads decreased, as a proportion of total body weight in ducks which died prior to Day 30, as compared with Zn-dosed ducks that survived.

Erythrocyte PCV values for Zn-dosed ducks decreased, on average, between Days 0 and 15, before increasing between Days 15 and 30. Mean values were lower for Zn- as compared with sham-dosed ducks at Days 15 and 30. Zn-dosed ducks had a higher mean reticulocyte count, and a greater number of individuals with immature and/or abnormal erythrocytes, than did sham-dosed mallards.

Mean total leucocyte counts were higher in Zn-dosed ducks, and in those Zn-dosed ducks
which had soil available. Zn-dosed ducks were characterized by a marked heterophilia and relative lymphopenia. Diet and diet x dose interaction effects on mean lymphocyte counts were detected.

Zn-dosed mallards evinced higher serum aspartate aminotransferase and lower alkaline phosphatase activity than sham-dosed ducks. Serum amylase activity activity was 25% higher in Zn-dosed ducks, however, this difference was not detectable at \( \alpha = 0.05 \). Serum phosphorus and uric acid concentrations were higher, and calcium, glucose, and total protein levels lower, in Zn-dosed ducks. Diet effects were detected for serum calcium, phosphorus, total protein, and uric acid concentrations.

Gross lesions observed in Zn-dosed ducks which died of Zn intoxication included pectoral muscle atrophy, typhlitis, intestinal enteritis, pericardial and/or serosal mineralization, and hepatic granulomas. Macroscopic changes tended to be less severe in ducks fed the commercial pelleted ration.

Dosing of 6-8 month-old, female, game-farm mallards with 8 No. 4 Zn shot resulted in reduced survival, behavioral signs of intoxication, loss of body weight, organ hypertrophy or atrophy, reduction in PCVs, and gross pathological changes. These signs were consistent those observed in mallards dosed with 6 No. 4 Zn shot of the same composition and maintained on a corn diet (Levengood et al. 1997). The effects of a more nutritionally-complete diet in provide protection from Zn toxicosis were neither dramatic nor conclusive.
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INTRODUCTION

Continued interest exists in the development of nontoxic substitutes for lead (Pb) shot pellets, amid dissatisfaction with ballistic properties and hardness of "steel" or iron (Fe) shot. In some areas steel shot has been prohibited due to timber industry concerns regarding damage to equipment used in the production of high-quality lumber, creating an additional demand for shot composed of softer materials. Currently, steel, bismuth/tin (Bi/Sn), and tungsten/iron (W/Fe) shot are approved as nontoxic shot in both the U.S. and Canada; in addition, the Canadian Wildlife Service has recently approved the use of tungsten/polymer shot.

Although a variety of formulations have been tested, nearly all Pb-shot substitutes have been deemed unacceptable due to toxicity, expense, availability of materials, or poor ballistics. Zinc shot is currently in use as a Pb shot substitute in parts of Europe, and Zn coatings of less than 0.002" thickness or less than 1.0 % of the weight of the pellet are approved in the U.S.

Although lighter in weight than Fe, the malleability of Zn makes it a popular alternative in Europe, where thin-walled barrels and expensive shotguns are widely used.

Several past studies have addressed the toxicity of Zn shot to waterfowl. Irby et al. (1967) tested the toxicity of 10 shot types on 1-year-old game-farm mallards, and found that mortality and weight loss in ducks dosed with 8 No. 6 Zn-coated Fe shot did not differ from that of controls. Eighteen-month-old drake mallards dosed with 8 No. 6 Zn shot comprised of 92% Zn, 0.16% Pb, trace Fe, and 7% undetermined components exhibited weight loss, behavioral anomalies, kidney histopathology (1 duck), high hepatic Fe concentrations, and reduced survival (Grandy et al. 1968). More recently, Levengood et al. (1997) conducted a 30-day acute toxicity
test of shot comprised of 98% Zn and 2% Sn, using 6-8 month-old male and female game-farm mallards. In their study, ducks dosed with 6 No. 4 Zn shot exhibited reduced survival, weight loss, changes in organ weights, anemia, ataxia and paresis, high tissue Zn levels and alterations in levels of other elements, and a variety of gross and microscopic lesions.

In contrast to the above studies, French et al. (1987) dosed 1 yr-old mallards with either 5 or 10 nearly pure (99.9%) No. 6 Zn shot. After 28 days the authors reported no gross lesions or abnormalities in either dosing group, and no histopathological changes in liver or kidneys of the lower dosing group were noted. The dosed ducks gained weight, exhibited normal liver Fe levels, and increased liver Zn concentrations.

Comparisons among studies must be made carefully, given differences in age, sex, dosing levels, shot composition, timing and length of studies, rate of shot voidance, and diet. Although the shot used in our study approached the purity of that utilized by French et al. (1987), our results were more similar to those of Grandy et al. (1968) who used shot containing Pb and other impurities. Differences in diet among these studies were readily apparent. Grandy et al. (1968) fed their ducks a mixture of commercial duck pellets and mixed grains for 9 months prior to the start of their study, at which time they were switched to cracked corn, quartz, and oyster shell grit. According to CWS protocol, mallards in our prior study (Levengood et al. 1997) were fed a nutritionally-deficient diet of shelled corn and had no access to grit beyond what was contained in their gizzards upon arrival at our facility. In contrast, French et al. (1987) fed their ducks a more balanced ration of wheat, barley, “turkey crumbs”, and grit.

Diet, including ingestion of soil and grit, can have a dramatic effect on Pb shot erosion and Pb absorption, retention, and excretion rates, and can be important in mitigating the toxic
effects of ingested Pb shot (Sanderson and Bellrose 1986). Dietary components such as Ca, P, and protein, along with the size and hardness of food items, are import in determining the susceptibility of ducks to Pb poisoning (Jordan and Bellrose 1950, 1951; Jordan 1968; Sanderson and Bellrose 1986).

Diet might be expected to play an even greater role in Zn than in Pb toxicosis in waterfowl, given the essential nature of Zn to living organisms, resistance of birds to high Zn concentrations, known antagonisms between Zn and elements such as Ca, copper (Cu), Fe, and P, and the influence of other dietary inhibitors, such as phytate, lignin, and hemicellulose, on Zn absorption (Becker and Hoekstra 1971; Underwood 1971; Eisler 1993; Walsh et al. 1994). The objective of the current study was to examine the influence of 4 diets (whole kernal corn and commercial duck ration, each with or without soil available) on the toxicity of Zn shot to game-farm mallards.

METHODS AND MATERIALS

The Acute Toxicity Study was conducted using 120 female wild-type game-farm mallards, 6 to 8 months of age, purchased from Whistling Wings, Hanover, Illinois. The ducks were transported from Hanover to Champaign, Illinois, by truck in crates on 13 May 1997.

Upon arrival at the Illinois Natural History Survey’s (INHS) facility in Champaign, the ducks were weighed and assigned to pens by removing each duck from a crate and matching it with a randomly-drawn numbered leg band. Each duck was then placed in a correspondingly-numbered pen. Ducks were randomly assigned to one of 2 dosing groups (dosed with 8 No. 4 Zn shot or sham-dosed), and to one of 4 dietary treatment groups using the Select Cases procedure.
of SPSS (SPSS, Inc. 1996a). Dietary treatments were: corn only (CORN); corn with soil available (CORN/SOIL); commercial duck pellets only (PELLETS); commercial duck pellets with soil (PELLETS/SOIL).

The consecutively-numbered, elevated, outdoor, 1 m² pens are constructed of vinyl-coated, 1-inch (25.4-mm) mesh, 14-gauge wire. A 10- x 40-yard pole barn without sides provided a roof over the pens. Individual metal trays were placed under each pen to catch droppings and aid cleaning.

Facilities for holding the ducks were inspected by several members of the Laboratory Animal Care Committee, University of Illinois, prior to the study. Committee members also visited the facilities several times during the study. Commercial duck pellets containing a minimum of 17.0% protein (Heinhold 17% Duck Finisher Pellet, Heinhold Feeds, Inc., Kouts, IN) were provided ad libitum during the 20-day acclimatization period. On the date of dosing, the pellets were removed, and whole-kernel corn or a 14% breeder ration was provided ad libitum for the duration of the study.

The 14% Duck Breeder Developer Pellet ration, provided to the PELLETS and PELLETS/SOIL groups, was comprised of corn, soybean meal, ground oats, wheat middlings, alfalfa meal, meat and bone meal, and various vitamin and mineral supplements. Guaranteed analysis was crude protein ≥ 14.0%, crude fat ≥ 2.0%, and crude fiber ≤ 8.0%. The mean dry weight concentrations of selected elements in 12 composite pellet samples, as determined by Inductively Coupled Argon-Plasma Emission Spectroscopy (ICP) analysis, were as follows: Ca, 106 ppm; Cu, 11.6 ppm; Fe, 256 ppm; P, 6617 ppm; Pb, < Method Detection Limit (MDL) of 2.94 ppm; Sn, < MDL of 2.55 ppm; Zn, 75.7 ppm.
The CORN and CORN/SOIL groups were fed whole kernal corn obtained at the University of Illinois Department of Animal Sciences’ feed mill. Crude protein levels in corn harvested in 1996 and analyzed by the Animal Sciences Laboratory averaged approximately 9.5% (L. Bauer, Dept. of Animal Sciences, UI, pers. commun.). The mean concentrations (DW) of selected elements in 12 composite corn samples, as determined by ICP analysis, were as follows: Ca, 250 ppm; Cu, 2.2 ppm; Fe, 59.7 ppm; P, 2248 ppm; Pb, < MDL of 2.93 ppm; Sn, < MDL of 2.55 ppm; Zn, 24.0 ppm.

Approximately 1200 pounds (545.5 kg) of soil was obtained at the Chautauqua National Wildlife Refuge near Havana, Illinois. Lake Chautauqua is a traditional waterfowl concentration area on the Illinois River floodplain in Mason County, Illinois. Soil was collected in an area predominated by wet Choctah loam, a floodplain soil composed of 7-27% clay, 28-50% silt, 23-52% sand, and 3-6% organic matter (Calsyn 1995).

Soil was partially air-dried, tilled with a garden tiller, and ground using a Linding TD-10 soil grinder to break up the larger clods. The average composition of 2 samples of the processed soil was 1.3% gravel, 71.5% sand, 24.0% silt, and 3.3% clay. The mean concentrations (DW) of selected elements in 10 soil samples, as determined by ICP analysis, were as follows: Ca, 4535 ppm; Cu, 7.0 ppm; Fe, 5946 ppm; P, 176 ppm; Pb, 13.4 ppm; Sn, < MDL of 2.28 ppm; and Zn, 17.7 ppm. Although hunting has been prohibited on the lake portion of the refuge since 1944 (Bellrose 1959), and the high rate of sedimentation occurring along the Illinois River would be expected to have made any spent lead shot long since unavailable, we washed and sieved 4 soil samples totaling 8.9 kg for shot pellets. The remaining particles were inspected both visually and radiologically, however, no pellets were detected. Additionally, no foreign shot pellets were
found in the gizzards of the ducks upon dissection. Soil was provided to the CORN/SOIL and PELLETS/SOIL groups in rubber feed tubs and was watered as necessary to maintain soil moisture.

The study began on 2 June 1997 (Day 0) when the ducks were weighed, bled for PCV determination, and dosed. Before dosing, the doses of 8 No. 4 (0.13" [3.30 mm] dia.) Zn shot were counted, weighed, and placed in individual vials in the laboratory. The type, number, and weight of shot were recorded on the top of each vial and on a data sheet for each duck. At dosing, the shot dose was matched with the corresponding duck. A small plastic funnel fitted with a plastic tube (3/8 inch [9.5 mm] outside diameter, 9 inches [22.9 cm] long) was inserted down the gullet and into the proventriculus. The tube was kept in a pail of water between dosings to facilitate insertion into the alimentary canal. The shot were poured into the funnel and flushed into the proventriculus with approximately 5 mL of water.

Body weights were recorded and blood samples for PCVs collected on Days 0, 15, and 30; additional blood was collected on Day 15 for analysis of hematology and serum biochemistry parameters. Blood was collected by venipuncture in heparinized microhematocrit capillary tubes for PCV determination, and in 3.0-mL syringes fitted with twenty-gauge, 1-inch (2.54-mm) needles to obtain samples for hematology and serum biochemistry determinations. Approximately 1 mL of whole blood was transferred to EDTA-treated Vacutainer® tubes for hematological analysis. An additional 1 1/2 - 2 mL of blood were transferred to untreated Vacutainer® tubes and set aside for one hour before centrifugation and harvest of serum for determination of selected biochemistries.

Hematological parameters included PCVs, reticulocyte counts, erythrocyte morphology,
total leucocyte count, leucocyte differential, leucocyte morphology, and thrombocyte counts. Microhematocrit tubes were spun for 5 minutes at 13,000-g force, and the PCV read using a Micro-Capillary Reader (Damon/IEC). Erythrocyte and leucocyte morphology was evaluated through microscopic examination of stained blood smears.

Differential leucocyte (WBC) counts were made by categorizing and counting the first 100 WBCs observed on a stained blood smear. The eosinophil Unopette system and a hemacytometer were used to obtain an absolute count of heterophils and eosinophils. The total WBC count was determined by correcting for WBCs other than heterophils and eosinophils as follows:

\[
\text{Total WBC/\mu L} = \frac{\# \text{ of cells counted in hemacytometer chamber} \times 1.1 \times 16 \times 100}{\% \text{ of heterophils + eosinophils}}
\]

Mean thrombocyte and reticulocyte counts were calculated by counting the number of each in 10 high power fields, excluding the highest and lowest counts, and averaging the remaining 8 fields. An actual thrombocyte count can be estimated as follows:

\[
\text{Estimated thrombocyte count/\mu L} = \frac{\# \text{ thrombocytes/8 fields}}{1000} \times 3,500,000
\]

Serum alkaline phosphatase, amylase, and aspartate aminotransferase activity, and Ca, glucose, P, total protein, and uric acid concentrations were determined using a Hitachi 705 Automated Chemistry Analyzer (Boehringer Mannheim Diagnostics). Reagents and methodologies were provided by BMD.

Detailed behavioral observations were recorded each morning (without reference to
dosing) and a cursory visit was made in the afternoon to note any changes in severity of signs, process any dead ducks, and ascertain whether any ducks might be candidates for euthanasia. Following Levengood et al. (1997), we modified the observations of Grandy et al. (1968) to rank affected individuals as exhibiting mild, moderate, or severe signs as follows:

**Mild**: signs may not be readily apparent; close observation reveals abnormal gait, with bird at least occasionally lifting its feet higher than normal when walking or running; may appear slightly lethargic; otherwise may appear normal.

**Moderate**: signs readily apparent; bird high-stepping as if walking on hot surface; easily noted difficulty in walking or running; trouble maintaining balance when disturbed and regaining it after a fall; may exhibit difficulty folding wings; may appear normal if undisturbed; will attempt to evade on approach.

**Severe**: bird cannot stand or maintain balance, or can do so only with great difficulty; no or only feeble attempt to evade on approach.

For reporting of observational data, ducks that exhibited mild signs for only 1 day during the course of the study were not recorded as exhibiting signs of toxicosis. This precaution reduced the possibility that the typical gait of ducks walking on the wire pens was confused with gait abnormalities indicative of Zn toxicosis. Also noted were the condition of feces (if atypical), whether each individual had apparently fed (noted by the presence or absence of spilled food on
the duck’s tray), and any other noteworthy observations.

The Zn-dosed ducks were fluoroscoped on Days 7 and 8 (30 each day) at the University of Illinois College of Veterinary Medicine's Large Animal Radiology Laboratory to confirm retention of 8 shot pellets. All ducks (including sham-dosed) were transported in poultry crates a distance of 1.6 km to the radiology lab. Sham-dosed ducks were not fluoroscoped. For fluoroscopy, each bird was restrained in a ½ gallon paper milk carton with a hole cut in the bottom, which allowed the head and neck to protrude. The 4-sided carton was turned 90° to provide dorsal, ventral, and lateral views, which facilitated determination of the number of pellets present. Ducks retaining fewer than 8 pellets were re-dosed to replace the missing pellets.

Analysis of chemical composition of the shot was conducted at the Illinois State Water Survey's Analytical Laboratory, Champaign, Illinois, using Inductively Coupled Argon Emission Plasma Spectroscopy (ICP). Ten randomly-selected pellets were composed of an average of 98% Zn and 2% Sn; other elements were essentially undetectable (< 0.1% each).

All surviving ducks used in the study were weighed and blood was collected from the cutaneous ulnar vein as scheduled on Day 15 and Day 30. Subsequently, the ducks were euthanized by decapitation and necropsied on Day 30 or 31, and the gizzards, livers, kidneys, pancreases, and gonads were examined, excised, weighed, and frozen.

A board certified anatomic veterinary pathologist (GLF) necropsied 24 of 29 ducks dying before Day 30 at the University of Illinois’ School of Veterinary Medicine’s Diagnostic Laboratory. The condition of all major tissues and organs was noted and the organs were weighed. The remaining 5 ducks, which succumbed prior to the end of the study, were examined by the first author (JML).
The necropsies on the ducks surviving to Day 30 were conducted in the Animal Autopsy Room of the Natural Resources Studies Annex on the campus of the University of Illinois. On Day 30 the pathologist performed post-mortems on 10 randomly-selected control ducks (5 corn and 5 pellet fed ducks). The remaining ducks were examined by project personnel on Days 30 and 31. These ducks were euthanized on Days 30 and 31 to insure that fresh specimens would be examined.

Statistical Methods

Kaplan-Meier survival functions were derived using the KM procedure of SPSS (SPSS, Inc. 1996b); all cases were specified as uncensored and functions of different treatment groups were compared using the Breslow, or generalized Wilcoxon, test. We examined variation in whole body and organ weights, shot and grit retention, hematological variables, and serum biochemistries using a randomized, 2 x 4 factorial, fixed effects ANOVA, using dose and diet as grouping factors; Tukey’s HSD test was used for pairwise comparisons among treatments. Independent-samples t tests were used to compare organ weights between surviving Zn-dosed ducks and those that died prior to Day 30. Normality was assessed with the Kolmogorov-Smirnov Statistic with Lilliefors Significance Correction, the skewness statistic, and visual examination of data distributions. Weights, hematological, and biochemistry parameters were \( \log_e \) or arcsine (PCV) transformed to improved the distribution of data and reduce heterogeneity of variances among treatment groups. These procedures were available through SPSS (SPSS Inc. 1996c).

Change in body weight was expressed as \( \{ (\text{body weight}_{ij} - \text{body weight}_{iu}) / \text{body weight}_{iu} \} \times 100 \), and change in PCV as \( \{ (\text{PCV}_{ij} - \text{PCV}_{iu}) / \text{PCV}_{iu} \} \times 100 \). The high mortality observed in
Zn-dosed ducks precluded a meaningful repeated measures analysis; changes in variables examined over time were compared descriptively. A probability level of $P < 0.05$ was accepted as significant. The serum glucose (5 mg/dL) and alkaline phosphatase (5 U/L) data for duck numbers 22 and 4, respectively, were omitted as outliers due to the extremity of these values. The PCV value (21%) for duck number 92 at Day 15 was also eliminated; this was a sham-dosed duck with PCVs of 59% and 56% on Days 0 and 15, respectively.

RESULTS AND DISCUSSION

Survival

All sham-dosed ducks survived to Day 30/31, when the ducks were euthanized. Twenty-nine of 60 (48.3%) Zn-dosed ducks died or were euthanized prior to Day 30. This percentage is similar to the mortality rate for male (9/20, 45.0%), but lower than that for female (16/20, 80.0%), mallards dosed with 6 No. 4 Zn shot (Levengood et al. 1997). Grandy et al. (1968) reported 20% mortality to 30 days in 15 male mallards dosed with 8 No. 6 Zn shot, and Gasaway and Buss (1972:1114) indicated that “severe mortality occurred after 30 days”, with 92% mortality to 60 days, in ducks fed 3,000 to 12,000 mg/kg Zn carbonate in their diet. In contrast, French et al. (1987) found no mortality to 28 days in mallards dosed with 5 or 10 pure (99.9%) No. 6 Zn shot pellets.

Mortality rates for ducks in each Zn-dosed dietary treatment group were as follows:

CORN, 53.3 %; PELLETS/SOIL, 53.3 %; CORN/SOIL, 46.7 %; PELLETS, 40.0 %. Survival functions did not differ among treatments within the Zn-dosed group ($X^2 = 0.04$ to 0.68, $P \geq 0.41$). Survival in female Zn-dosed ducks averaged 22.7 days, as compared with mean survival times of 18 and 23 days in female and male mallards, respectively, dosed with 6 No. 4 Zn shot
(Levengood et al. 1997), and 20 days reported by Grandy et al. (1968) for 18-month old drake mallards dosed with 8 No. 6 Zn shot.

Mean number of days survived for ducks in each Zn-dosed dietary treatment group were as follows: CORN/SOIL, 24.1 days; PELLETS, 23.8 days; PELLETS/SOIL, 21.5 days; CORN, 21.2 days. We did not detect a diet treatment effect on the mean number of days survived within the Zn-dosed cohort ($F_{3,56} = 0.49, P = 0.69$). The shortest survival time for an individual duck in the current study was 8 days (PELLETS/SOIL group), as compared with 4 days in a mallard dosed with 8 No. 2 Pb (Sanderson et al. 1992) and 5 days in a mallard dosed with 6 No. 4 Zn shot (Levengood et al. 1997).

Retention and Dissolution of Shot

Twenty-nine ducks were observed to have fewer than 8 shot at fluoroscopy (7-8 days after dosing); > 8 shot were recovered from the gizzards of 7 of these ducks. Difficulty in accurately determining when 8 shot were present may have resulted from a combination of the relatively large dose of shot and the fluoroscopy technique, which did not involve continuous turning of the duck while attempting to ascertain the number of pellets present, as had been done in the past. Assuming that the actual number of shot at fluoroscopy equaled the number of shot recovered from the gizzard minus the number of shot redosed, there were 22 ducks with < 8 shot at fluoroscopy. The shot retention rate to Day 7, correcting for over-dosing, was 89.6 % (430/480), or an average of 7.2 pellets/duck. One duck was observed with 2 pellets in its gizzard and 4 in its lower gut; although the pan below this individual’s pen and feces it contained were checked for several days, the fate of the 4 pellets is unknown. This duck was redosed with 6 pellets after fluoroscopy.
After necropsy, the contents of the gizzard, proventriculus, and a short length of intestine were examined for shot. The linings of gizzards from Zn-dosed ducks were brittle and ranged from dark brown to green or yellowish-green, as was previously reported for mallards dosed with Zn shot (Levengood et al. 1997). Severe gizzard erosion was observed in a gray-headed chachalaca (*Ortalis cinereiceps*) that had ingested a copper-plated Zn coin (Droual et al. 1991), and Sanderson and Bellrose (1986:8) indicated that the gizzard lining of Pb-poisoned waterfowl is often “dark, soft, decayed, easily eroded, inflamed, corroded, and incomplete”.

The retention rate for all shot dosed was 63.6% (343/539), which was considerably lower than that for mallards dosed with 6 No. 4 Zn shot (91.7%) of the same composition (Levengood et al. 1997). Grandy et al. (1968) found that only 3 of 15 Zn-dosed mallards retained any shot to 30 days, with none retaining the original dose of 6 pellets. French et al. (1987) reported retention rates of 98% and 50%, respectively, 28 days after dosing in mallards dosed with 5 or 10 No. 6 Zn shot.

The mean number of shot retained/duck in this study was the same (5.8 pellets) as that reported for mallards dosed with 6 No. 4 Zn shot (Levengood et al. 1997). We did not detect any diet effects on the number of shot recovered/gizzard ($F_{3,55} = 1.1, P = 0.36$).

The gizzards of sham-dosed ducks contained more grit ($\bar{x} = 2.8$ g, SE = 0.16) at necropsy than those of Zn-dosed ducks ($\bar{x} = 2.1$ g, SE = 0.17) ($F_{1,112} = 10.6, P = 0.002$). If those ducks that died prior to Day 30 are excluded, because their food and soil consumption ceased, the dose effect disappears (the means become identical). The CORN/SOIL (2.9 g, SE = 0.23) and PELLETS/SOIL (3.0 g, SE = 0.26) had a larger amount of grit in their gizzards at necropsy, on average, than the CORN (1.9 g, SE = 0.19) and PELLETS (2.1 g, SE = 0.22) groups ($F_{3,112} = 7.0$, 13
The mean dissolution rate for shot in all Zn-dosed ducks retaining 8 pellets (n= 21) was 0.0201 g/day, for a mean total dissolution of 47.1% of the original shot weight. This compares with a dissolution rate of 0.017 g/day and mean dissolution of 53.3% of the shot weight for mallards dosed with 6 No. 4 Zn shot (Levengood et al. 1997).

The mean dissolution rate for shot in ducks that retained 8 shot and survived to Day 30 (n= 10) was 0.0245 g/day, for a total dissolution of 75.1% of the original shot weight, compared with a dissolution rate of 0.0160 g/day and a total loss of 21.7% of the shot weight in ducks that retained 8 shot and died prior to Day 30 (n= 11). Levengood et al. (1997) reported a dissolution rate of 0.022 g/day and dissolution of 88.1% of the original shot weight for mallards dosed with 6 No. 4 Zn shot and surviving to Day 30.

Dissolution rates by treatment for shot in all Zn-dosed ducks retaining 8 pellets were as follows: CORN/SOIL (0.025 g/day, n= 3) > PELLETS (0.022 g/day, n= 6) > CORN (0.020 g/day, n= 7) > PELLETS/SOIL (0.016 g/day, n= 5). For all Zn-dosed ducks retaining 8 pellets and surviving to Day 30 dissolution rates by treatment were as follows: CORN/SOIL (0.020 g/day, n= 1) > CORN (0.017 g/day, n= 5) > PELLETS (0.015 g/day, n= 1) > PELLETS/SOIL (0.014 g/day, n= 4). Similarly, dissolution rates by treatment for all Zn-dosed ducks retaining 8 pellets and surviving < 30 days were as follows: CORN/SOIL (0.027 g/day, n= 2) > CORN (0.026 g/day, n= 2) > PELLETS (0.023 g/day, n= 5) = PELLETS/SOIL (0.023 g/day, n= 1).

Small and disparate samples sizes did not allow a meaningful statistical comparison of pellet dissolution rates across treatment groups, however, there was an obvious trend toward higher rates in the groups fed corn than those provided the pelletized ration. Interestingly, in each
instance the CORN/SOIL group dissolved shot at the highest rate, whereas ducks in the
PELLETS/SOIL cohort had the lowest rate of dissolution.

Behavioral Abnormalities

Signs of zinc toxicosis observed were consistent with the observations of Grandy et al.
(1968), Gasaway and Buss (1972), and/or Levengood et al. (1997). These signs included ataxia,
paresis, and reduction or cessation of food intake. Behavioral signs of Zn toxicosis were
initially noted in 5 ducks on Day 4 of the study. Mortality first occurred on Day 8, 1 day after
that duck began showing mild behavioral signs of intoxication, and 2 days after it was noted
passing blood. Levengood et al. (1997) noted signs of intoxication on Day 4 and mortality on
Day 5 in ducks dosed with 6 No. 4 Zn shot of the same composition as in the current study.

Forty-one of 60 (68.3%) Zn-dosed ducks displayed ataxia and/or paresis during the
experiment. Eighteen of 20 (90.0%) female ducks dosed with 6 No. 4 Zn shot of the same
composition as in the present study exhibited behavioral signs during a 30-day acute toxicity test
(Levengood et al. 1997). Grandy et al. (1968) detected behavioral anomalies in 12 of 15 (80%)
male mallards dosed with 8 No. 6 Zn shot. In their study of the effects of dietary Zn carbonate
on mallards, Gasaway and Buss (1972) noted severe paralysis after 20 days, with the onset being
most rapid in the group receiving the lowest dosage.

Within each diet group (corn vs. pellets), fewer ducks exhibited behavioral signs (> 1
observation day) of Zn intoxication when soil was available (Fig. 1). The number of ducks
exhibiting signs, however, did not differ significantly across the 4 treatments ($\chi^2 = 0.87, 0.75 < P
< 0.90$).

Zinc-dosed ducks typically exhibited a cessation of feeding before showing moderate
signs of toxicosis. All but 1 duck that displayed only mild signs of intoxication survived to Day 30. Ten of the ducks that exhibited moderate to severe signs survived, and mild signs persisted to Day 29 or 30 in only 3 of these individuals. Some Zn-dosed ducks went through periods of improvement and deterioration over the course of a few days or even several hours. Levengood et al. (1997) reported that none of the ducks showing behavioral abnormalities in a 30-day acute toxicity test using Zn shot completely recovered from the effects of Zn-intoxication. Grandy et al. (1968) reported that 1 of 3 ducks retaining shot to the end of their study showed no signs of intoxication, whereas another exhibited signs for 5 days but had “fully recovered” by Day 30.

Three ducks passed blood on one occasion each. Although all 3 died subsequent to passing blood, this condition did not persist until death. As noted by Levengood et al. (1997), other signs of toxicosis included dark or bright green feces, oral cavity pallor, diarrhea, foul-smelling excreta, a drooping or tucked tail, clacking of the bill and other uncontrolled movements of the head, and evasive behavior indicative of diseased waterfowl.

**Body Weight**

All ducks as a group lost weight (\( \bar{x} = 68 \text{ g} \)) during the 20-day acclimatization. Sham-dosed ducks weighed more than Zn-dosed ducks at Days 0, 15, and 30 (Table 1). Presumably the difference at Day 0 represented a Type I Error, as no difference would be expected since ducks were randomly assigned to a treatment. The proportional difference ([1 - \( \frac{\bar{x} \text{ weight Zn-dosed}}{\bar{x} \text{ weight sham-dosed}} \)] \times 100) in mean body weight between Zn- and sham-dosed ducks was considerably less at Day 0 (3.9%), as compared with Day 15 (17.8%) and Day 30 (13.6%).

We detected a diet effect at Day 30; post-hoc testing revealed that the mean body weight of the PELLETS group (\( \bar{x} = 1.03 \text{ kg}, \ SE = 0.04 \)) was significantly greater (\( P = 0.05 \)) than that of
the CORN/SOIL group (\( x = 0.92 \) kg, SE= 0.03). Within the Zn-dosed cohort, mean body weight was greatest in the PELLET group at Days 15 and 30, and lowest in the CORN (Day 15) and CORN/SOIL groups (Day 30) (Fig. 2), however, we did not detect significant interactions between dose and diet at Day 15 or 30.

Zn-dosed ducks lost weight, on average, between Days 0 and 15, and gained weight between Days 15 and 30 (Table 1). These data included body weights for ducks that died between Day 0 and 15 and Day 15 and 30, respectively. Variability in weight did not change remarkably throughout our study.

Zn-dosed ducks lost an average of 16% of their body weight between Day 0 and Day 15 or death (if < Day 15), compared with a loss of 1% for sham-dosed ducks. Zn-dosed ducks that died prior to Day 15 lost 32% of their body weight on average between Day 0 and death, compared with a mean loss of 11% between Day 0 and Day 15 for Zn-dosed ducks that survived to Day 15.

Zn-dosed ducks lost 9% of their body weight between Day 0 and Day 30 or death (if < Day 30), compared with a mean weight gain of 1% for sham-dosed ducks. Zn-dosed ducks that died prior to Day 30 lost 33% of their body weight, on average, between Day 0 and death, compared with a mean loss of 1% between Day 0 and Day 30 for Zn-dosed ducks that survived to Day 15.

Sham-dosed ducks showed little change in weight from Day 0 to 15 and from Day 15 to 30 (Table 1). This pattern was similar to that reported by Sanderson et al. (1997a) for Bi-, Fe-, and O-dosed mallards.

The dramatic loss of body weight observed for Zn-dosed ducks in our study was typical...
of that detected in studies involving Zn- or Pb-dosed waterfowl. Levengood et al. (1997) found that male and female mallards dosed with 6 No. 4 Zn shot lost an average of 33% and 40% of their body weight, respectively, between Day 0 and death. Sanderson and Bellrose (1986) reported that mallards dying of chronic Pb-poisoning typically lose 40-60% of their body weight.

Mean weight loss to 30 days in mallards fed Zn carbonate along with a chicken developer - turkey finisher diet ranged from 17-44%, with females losing a greater proportion of body weight than males (Gasaway and Buss 1972). Grandy et al. (1968) reported that mean weight loss in male mallards dosed with 8 No. 6 Zn pellets and placed on a corn and grit diet was 33% for 3 birds that died, and 22% in those surviving 30 days. In contrast, French et al. (1987) found that mallards dosed with 5 or 10 No. 6 Zn shot and kept on a varied diet of small grains, commercial feed, and grit gained weight.

**Organ Weights**

**Liver**

Mean liver weight was significantly greater in sham-dosed ducks, as compared with Zn-dosed ducks (Table 2). We did not detect significant diet or dose x diet interaction effects for liver weight. The liver represented a similar proportion of total body weight in Zn- (2.1%) and sham-dosed ducks (1.9%).

Mean liver weight was significantly greater in Zn-dosed ducks that survived to Day 30 than in those that did not (Table 2). The liver represented a similar proportion of total body weight in Zn-dosed ducks surviving <30 days (2.2%) and those surviving to Day 30 (1.9%).

**Pancreas**

Pancreas weight averaged significantly greater in sham- than in Zn-dosed ducks (Table
We did not detect significant diet or dose x diet interaction effects for pancreas weight. Mean pancreas weight represented a greater proportion of total body weight in sham-dosed ducks (0.26%) as compared with Zn-dosed ducks (0.21%).

Pancreas weight was significantly greater in Zn-dosed ducks that survived to Day 30 than in those that did not (Table 2). The pancreas represented the same proportion of body weight in those Zn-dosed ducks surviving < 30 days and those surviving to Day 30 (0.21%).

**Kidneys**

Average kidney weight was significantly greater in Zn- than in sham-dosed ducks (Table 2). The kidneys represented a greater proportion of total body weight in Zn-dosed (0.88%) as compared with sham-dosed ducks (0.60%). We also detected a diet effect on kidney weight, with the kidneys of ducks in the PELLETS (x̄ = 7.3 g, SE = 0.2) and PELLETS/SOIL (x̄ = 7.3 g, SE = 0.3) groups averaging greater than those of ducks in the CORN (x̄ = 5.7 g, SE = 0.3) and CORN/SOIL (x̄ = 5.6 g, SE = 0.3) groups.

Kidney weight was significantly greater in Zn-dosed ducks that died prior to Day 30 than in those that survived (Table 2). The kidney represented twice the proportion of body weight in those Zn-dosed ducks surviving < 30 days (1.2%) and those surviving to Day 30 (0.6%).

**Gonads**

Mean gonadal weights were significantly greater in sham-dosed than in Zn-dosed ducks (Table 2). The gonad represented more than twice the proportion of total body weight in sham-dosed (0.47%) as compared with Zn-dosed ducks (0.20%). We did not detect significant diet or dose x diet interaction effects on gonad weight.

Gonad weight was significantly greater in Zn-dosed ducks that survived to Day 30 than in
those that did not (Table 2). The gonad represented a much larger proportion of body weight in Zn-dosed ducks surviving to 30 days (0.3%) than in those that did not (0.09%).

**Gizzards**

Mean gizzard weights were significantly greater in sham-dosed than in Zn-dosed ducks (Table 2). The gizzard represented the same proportion of total body weight in sham- and Zn-dosed ducks (2.5%). We also detected a diet effect on gizzard weight (Table 2), with the gizzards of ducks in the CORN ($x = 21.3$ g, SE = 1.0) group weighing significantly less than those of ducks in the PELLETS ($x = 25.6$ g, SE = 1.3) group.

Gizzard weight was significantly greater in Zn-dosed ducks that survived to Day 30 than in those that did not (Table 2). The gizzard represented a larger proportion of body weight in Zn-dosed ducks surviving to 30 days (2.8%) than in those that did not (2.2%).

**Discussion of Organ Weights**

In the present study the livers, pancreases, gonads, and gizzards were reduced, and kidneys enlarged, in Zn-dosed ducks relative to sham-dosed controls. The pancreas and gonads represented a greater proportion of body weight in sham-dosed ducks, whereas the kidneys accounted for a greater percentage of total body weight in Zn-dosed mallards. The livers, pancreases, gonads, and gizzards were lighter, and the kidneys were heavier, in ducks that died as a result of Zn intoxication than in those which survived 30 days. In addition, the kidneys increased, and gizzards and gonads decreased, as a proportion of total body weight in ducks, which died prior to Day 30, as compared with those that survived. Although no diet x dose interaction effects were detected, there was a trend towards heavier organs in those Zn-dosed ducks fed the pelletized ration (Fig. 3).
According to Sanderson and Bellrose (1986), the organs of Pb-poisoned waterfowl may be reduced or enlarged at death, depending on the stage and nature of the toxicosis. Sanderson and Irwin (1976) suggested that changes in liver size in Pb-intoxicated ducks were confounded by differing rates of food consumption among seasons and between sexes, diet, length of survival, and anorexia. Levengood et al. (1997) found that the kidneys were heavier, and the pancreases and gizzards lighter, in Zn-dosed mallards, as compared with Fe-dosed controls. They also reported that the livers (males) and kidneys of ducks that died from Zn intoxication were heavier, the gonads and gizzards lighter, and the livers, kidneys, and pancreases represented a greater proportion of total body weight, as compared with surviving Zn-dosed ducks. Gasaway and Buss (1972) found that ducks fed dietary Zn experienced significant reductions in the weight of the pancreas, liver, and gonad, compared with controls, whereas the kidneys represented a larger proportion of body weight. van der Zee et al. (1985) noted liver and kidney hypertrophy in a captive Nicobar pigeon (Caloenas nicobarica) that had ingested Zn fragments.

Hematology

PCV

Mean PCVs changed little in sham-dosed ducks over the course of the study (Table 3). Mean PCV values decreased in surviving Zn-dosed ducks between Days 0 and 15, before increasing between Days 15 and 30. Variability in PCVs increased throughout the study in Zn-dosed ducks, and was more than 3 times as variable as in sham-dosed ducks at Days 15 and 30. Mean PCV values were significantly lower in Zn-dosed, as compared with sham-dosed ducks, at Days 15 and 30 (Table 3). The lowest PCV value we documented was 13%, recorded for each of 2 Zn-dosed ducks just prior to euthanasia. We did not detect significant diet or dose x diet
interaction effects on PCVs. Mean PCV values in Zn-dosed ducks were highest in the PELLETS/SOIL, and lowest in the CORN/SOIL, groups at Day 15, and highest in the CORN, and lowest in the PELLETS/SOIL, groups at Day 30 (Fig. 4).

PCVs decreased by an average of 22% in Zn-dosed ducks and 4% in sham-dosed ducks, between Days 0 and 15. Individual values ranged from -76% to +8% and -30% to +13% in Zn- and sham-dosed mallards, respectively. PCVs decreased by an average of 8.9% in Zn-dosed ducks and < 1% in sham-dosed ducks, between Day 0 and 30. Individual values ranged from -74% to +32% and -22% to +24% in Zn- and sham-dosed mallards, respectively.

Immature and abnormal erythrocytes

The mean reticulocyte count was significantly higher in Zn- (x = 15.8/µL, SE = 1.3, n= 45) as compared with sham-dosed (x = 7.7/µL, SE = 0.4, n= 53) ducks (F1,90= 38.5, P<0.001). Individual reticulocyte counts ranged from 3 to 35 /µL and 4 to 18/µL in Zn- and sham-dosed mallards, respectively. We did not detect significant diet or dose x diet interaction effects on reticulocyte counts. Notable to marked polychromasia was observed in the blood of 11 of 45 (24%) Zn-dosed ducks; polychromasia was not remarkable in sham-dosed ducks. Basophilic or polychromatic prorubricytes were detected in the blood of 18 of 45 (40%) Zn- and 5 of 53 (9%) sham-dosed mallards.

Poikilocytosis was not noted in sham-dosed ducks but was observed in 11 Zn-dosed ducks (0 sham-dosed), and was judged as marked in 5 of these individuals. Binucleated erythrocytes were noted in one Zn-dosed individual.

Thrombocytes

We did not detect significant treatment effects on mean thrombocyte counts (x = 5.2/HPF,
SE= 0.2, n= 98). Individual values ranged from 2 to 15 /HPF. Immature thrombocytes were noted in the blood of 2 Zn-dosed ducks.

**Leukocytes**

The mean total leukocyte (WBC) count was significantly higher in Zn- as compared with sham-dosed ducks (Table 4). We also detected a significant dose x diet interaction effect on total WBC counts. For Zn-dosed ducks, WBC counts were higher for treatment groups, which had soil available, both within and between diets (Fig. 5). WBC counts in sham-dosed ducks were lower for treatment groups, which had soil available, both within and between diets. Individual WBC counts ranged from 7,270 to 99,365 /µL and 3,377 to 33,318 /µL in Zn- and sham-dosed ducks, respectively.

Zinc-dosed ducks had a higher proportion of heterophils and lower proportion of lymphocytes, relative to sham-dosed ducks (Table 4). The proportions of monocytes and eosinophils were similar between dosing groups. The mean heterophil count was significantly greater in Zn- as compared with sham-dosed ducks (Table 4). Individual heterophil counts ranged from 3,907 to 93,403 /µL in Zn-dosed ducks and 1,971 to 23,056 /µL in sham-dosed ducks. We did not detect significant diet or diet x dose interaction effects on mean heterophil counts. Toxic changes were detected in the heterophils of 11 of 45 (24%) Zn-dosed ducks, as compared with only one of 53 (2%) sham-dosed mallards. The severity of these changes was ranked as +1 in 2, +2 in 7, and +3 in 2 Zn-dosed mallards.

The mean lymphocyte count was lower in Zn- as compared with sham-dosed ducks (Table 4), however, this difference was not significant (P=0.09). Individual lymphocyte counts ranged from 2,106 to 12,996 /µL in Zn-dosed ducks and 878 to 22,657 /µL in sham-dosed ducks.
We detected a diet effect on lymphocyte counts; post hoc testing revealed that the mean lymphocyte count was lower in the PELLETS/SOIL group ($\bar{x} = 5078/\mu L$, SE = 420, n = 26) than in the CORN ($\bar{x} = 7540/\mu L$, SE = 1044, n = 20) or PELLETS ($\bar{x} = 7284/\mu L$, SE = 489, n = 26) groups. We also detected a significant dose x diet interaction effect on lymphocyte counts (Table 4). For Zn-dosed ducks, mean lymphocyte counts were highest in the PELLETS group, and lowest in the CORN group (Fig. 6). In sham-dosed ducks, the mean lymphocyte count was highest in CORN treatment group (although the SE was 2 - 3 times higher than the other groups), and lowest in the PELLETS/SOIL group. Reactive lymphocytes were observed in the blood of 14 of 45 (31%) Zn- and 11 of 53 (21%) sham-dosed ducks.

**Discussion of hematological parameters**

Severe anemia is common in Pb-poisoned waterfowl (Sanderson and Bellrose 1986), as well as in Zn-intoxicated vertebrates (Underwood 1971; Eisler 1993; Walsh et al. 1994). PCV values were greatly reduced in Zn-intoxicated mallards in our study, as well as in a 30-day acute test conducted by Levengood et al. (1997). PCV values in undosed, farm-raised mallards typically average about 45% - 46% (Ringelman et al. 1993; Duncan 1997; Levengood et al. 1997; Sanderson et al. 1997a,b), although PCVs in ducks may vary according to season (Shave and Howard 1976), molt stage (Driver 1981), and age (Kocan and Pitts 1976). Campbell (1988) considered PCVs of less than 35% in caged birds as indicative of anemia. In the present study, PCVs were below 35% in 16 of 50 (32%) Zn-dosed ducks at Day 15 or at the time of euthanasia (if occurring before Day 15). In contrast, PCVs were below 35% in only 4 of 35 (11%) Zn-dosed ducks at Day 30 or at the time of euthanasia (if occurring between Days 15 and 30). Anemia resulting from Zn toxicosis has been attributed to associated Cu and Fe deficiencies. Zn
intoxication can lead to faulty hematopoiesis and shortened erythrocyte life span due to Zn-mediated Cu deficiencies and Zn-Fe interactions.

Increases in reticulocyte counts, immature erythrocytes, and the degree of polychromasia are regarded as indicators of an erythrocyte regenerative response (Campbell 1994; Fudge 1997). Duncan (1997) considered increased reticulocyte counts, coupled with increased PCVs (following an initial drop in values), in Pb-dosed mallards to be indicative of a sufficient bone marrow response to Pb-induced anemia. We detected a higher mean reticulocyte count, increased polychromasia and incidence of immature erythrocytes, and lower mean PCV values in Zn-dosed ducks at Day 15, relative to controls, followed by an increase in the mean PCV in Zn-dose ducks at Day 30. Although the increase in the mean PCV in Zn-dosed ducks between Days 15 and Day 30 to some degree resulted from fewer severely-intoxicated ducks in the later sample, increased values for some individuals between Day 15 and 30 were noted.

The mean total WBC count in sham-dosed ducks in our study (14,523/µL) was similar to that reported by Fairbrother and O’Loughlin (1990) for captive adult male mallards (14,350/µL (mm³). The average total WBC count in Zn-dosed ducks (22,608/µL) was higher than the largest mean value documented by Duncan (1997) for Pb-dosed mallards (19,394/µL); our highest value (99,365/µL) exceeded that (51,200/µL (mm³) provided by Hemm and Carleton (1967) in their review of duck hematology. In comparison, average leucocyte counts ranged from 5,330 - 26,470/ µL (mm³) in drake mallards pre-, during, and post-remige molt, with values declining over the latter 2 periods (Driver 1981).

Leukocyte counts in Zn- and sham-dosed ducks were comprised of higher proportions of heterophils than lymphocytes. A broad range of values for the relative proportions of
lymphocytes and heterophils in mallards has been reported (see Hemm and Carleton 1967; Driver 1981; Fairbrother and O'Loughlin 1990; Ringelman et al. 1993; Duncan 1997). In the absence of disease, this variation may be attributable to the physiological state of the animal, as influenced by age (Hemm and Carleton 1967, Fairbrother and O'Loughlin 1990), molt stage (Driver 1981), gender (Duncan 1997), and seasonality (Duncan 1997).

Zn-dosed mallards evinced pronounced heterophilia and a concurrent mild lymphopenia. Heterophilias can be indicative of infectious and inflammatory diseases as well as toxicities are often the cause of leucocytosis, the magnitude of which often reflects the extent of the inflammatory response. Slight heterophilia in sham-dosed ducks would be consistent with the mild inflammatory changes considered normal for game-farm mallards. Marked heterophilia is consistent with gross lesions in this study, as well as gross and microscopic lesions reported by Levengood et al. (1997) for mallards dosed with 6 No. 4 Zn shot and maintained on a corn diet.

Fairbrother and O'Loughlin (1990) found a higher proportion of lymphocytes (54-68%) than heterophils (27-38%) in undosed male and female mallards from pre-egg laying through the post-reproductive period. Lymphocytes represented 37.0 to 55.9%, and heterophils 36.3 to 50.4%, of the total WBC count in captive mallards before, during, and after the remige molt (Driver 1981), with a slight preponderance of heterophils recorded prior to pre-basic molt, during remige molt (n= 1), and during remige development. With the exception of 1 outlier (our interpretation), Duncan (1997) reported a greater proportion of heterophils (55-94%) than lymphocytes (6-42%) in female mallards across dosing groups and time periods.

**Serum Biochemistry**

Zn-dosed ducks had higher mean serum aspartate aminotransferase (AST) activity and
phosphorus (P) and uric acid (UA) concentrations, and lower alkaline phosphatase (ALP) activity and calcium (CA), glucose (GLU), and total protein (TP) concentrations, than sham-dosed ducks (Table 5). Zn-dosed ducks experienced nearly 10- and 4-fold increases in mean AST activity and UA concentration, respectively, as compared with sham-dosed ducks. Although amylase (AMYL) activities were 25% higher in Zn-dosed mallards, this difference between dosing groups was not significant ($P = 0.10$).

Diet effects were detected for mean CA, P, TP, and UA concentrations (Table 5). Post-hoc testing revealed that mean serum CA and UA levels were higher in the PELLETS/SOIL group (CA- $\bar{x} = 16.6$ mg/dL, SE= 1.5; UA- $\bar{x} = 16.4$ mg/dL, SE= 5.6; n= 26) than in the CORN group (CA- $\bar{x} = 11.6$ mg/dL, SE= 0.4; UA- $\bar{x} = 6.0$ mg/dL, SE= 2.0; n= 24). Phosphorus concentrations in the PELLETS/SOIL ($\bar{x} = 7.1$ mg/dL, SE= 0.8, n= 26) group averaged higher than in each of the other treatment groups ($\bar{x} = 4.6$ to 5.3 mg/dL, SE= 0.4 to 0.6, n= 24 to 27). Mean TP concentrations were marginally greater ($P = 0.057$) in the PELLETS ($\bar{x} = 4.4$ g/dL, SE= 0.2, n= 26) group, as compared with the CORN/SOIL cohort ($\bar{x} = 3.9$ g/dL, SE= 0.2, n= 27).

Although we did not detect any significant dose x diet interaction effects on serum biochemistry parameters, mean enzyme activity levels and metabolite concentrations in Zn-dosed ducks were highest in either of the pellet-fed cohorts for 6 of 8 parameters examined (Fig. 7); means were highest in the PELLETS/SOIL group in 5 of the 6 cases. Mean CA, P, and UA levels were higher in both corn- and pellet-fed, Zn-dosed ducks, which were provided soil, as compared with those that did not have soil available. Mean GLU levels were higher in corn-fed ducks in both dosing groups. Trends involving mean enzyme or metabolite levels among diet treatments in sham-dosed ducks were closely reflective of those in Zn-dosed ducks in 4 of the
serum biochemistry parameters examined (ALP, GLU, TP, and UA).

Discussion of serum biochemistries

Alkaline phosphatase

ALP, a Zn-dependent enzyme, liberates inorganic phosphate from organic phosphate compounds (Strand 1978). Zn deficiency results in reduced ALP levels and activity (Underwood 1971; Prasad 1979; Kirchgessner and Roth 1980; Hochleithner 1994). The mean ALP activity level in Zn-dosed ducks at Day 15 of our study was higher than the range of means provided by Fairbrother et al. (1990) for hen mallards at various reproductive stages, and lower than plasma values for W/Bi/Sn- and sham-dosed mallards (sexes combined) in the autumn (Ringelman et al. 1993). Mean ALP activity in sham-dosed ducks was higher than Fairbrother et al. (1990) and Ringleman et al. (1993) reported for farm-raised mallards, and was more variable than in Zn-dosed ducks. Although elevated ALP activity may be associated with diseases of bone and liver (Cornelius 1989; Hochleithner 1994), ALP levels are also known to increase during egg-laying (Hochleithner 1994; Fairbrother et al. 1990), and higher activity levels in sham-dosed than in Zn-dosed ducks likely reflect the reproductive status of the respective cohorts. Gonadal development was arrested in Zn-dosed ducks (see section on gonadal weights), and only one Zn-dosed duck was known to have laid any eggs during our 30-day study.

Amylase

Mean serum AMYL activities in Zn- and sham-dosed mallards fell within the range of values for mallards from the pre-egg laying through postreproductive stages (Fairbrother et al. 1990). Although Fairbrother et al. (1990) found AMYL activity levels to be highest during egg
laying, the mean AMYL value was 25% higher in Zn- than in sham-dosed ducks in our study. AMY catalyzes the hydrolysis of complex carbohydrates, and the highest activities are found in the pancreas and duodenum (Kramer 1989). Elevated plasma or serum AMYL activity associated with pathological conditions is considered indicative of pancreatitis (Hochleithner 1994; Brobst 1989) or enteritis (Hochleithner 1994). Levengood et al. (1997) found Zn-intoxicated ducks characterized by pancreatic apoptosis, typhlitis, and enteritis involving the small and large intestines; clinical signs and gross pathological changes in Zn-intoxicated ducks were essentially identical to those observed in the current study.

Aspartate aminotransferase

AST, an enzyme involved in amino acid and carbohydrate metabolism, catalyzes the transamination of L-aspartate and 2-oxoglutarate to oxaloacetate and glutamate (Kramer 1989). Increased AST activity may reflect liver or muscle tissue damage (Cardinet 1989; Hochleithner 1994; Fudge 1997). Activities of 300-15,000 U/L are indicative of liver, muscle, or heart damage in avian species (Fudge 1997), and Hochleithner (1994) considered levels > 230 U/L in avian species as abnormal. AST activity in Zn-dosed ducks was greatly increased compared with sham-dosed mallards. The mean value approached, and activity in 12 individuals exceeded, the 230 U/L threshold. Although creatinine kinase activity levels are necessary to rule out muscle damage as the cause of increased AST levels, pathologic conditions involving the liver, heart, and pectoral muscles have been noted in Zn-dosed ducks (Levengood et al. 1997; this study).

Ca

Serum Ca concentrations increase during egg production, and mean concentrations in our study were higher in sham-dosed ducks, which, as a group, laid throughout the 30-day study.
Duncan (1997) detected a treatment x gender effect on serum Ca levels, with female Pb-dosed mallards exhibiting higher concentrations than female sham- and Bi-dosed ducks, whereas Ringelman et al. (1993) found higher plasma Ca concentrations in sham-dosed ducks than in those dosed with W-Bi-Sn shot. Levengood at al. (1997) documented lower plasma Ca concentrations in Zn- than in Fe-dosed mallards at Day 15 of a 30-day study, whereas no dose effects were detected for plasma (Sanderson et al. 1997a) or whole blood (Sanderson et al. 1997b) from mallards dosed with 0, Fe, or Bi shot. Mean serum Ca levels in the present study were within the range of means documented by Fairbrother et al. (1990) and Duncan (1997).

Although mean Ca concentrations were higher in corn (250 ppm DW) than in the pelletized ration (106 ppm DW), concentrations in the soil provided to the ducks were considerably higher than either. Thus, it might be expected that serum Ca levels should be higher in the both groups provided soil, as compared with those that were not. Although Ca levels were significantly higher in the PELLETS/SOIL ($\bar{x} = 16.6$ mg/dL) group than in the CORN ($\bar{x} = 11.6$ mg/dL) cohort, we cannot explain the lack of such a relationship between ducks provided the PELLETS/SOIL diet and those fed only PELLETS ($\bar{x} = 14.7$ mg/dL), or between those fed CORN/SOIL ($\bar{x} = 12.5$ mg/dL) and the CORN and PELLETS cohorts. Perhaps interactions between Ca and other dietary components were responsible for the absence of a pattern consistent with diet Ca levels alone.

Glucose

Although serum GLU concentrations were higher in sham-dosed than in Zn-dosed mallards, mean values in both groups were consistent with average values reported in other studies (Fairbrother et al. 1990; Ringelman et al. 1993; Duncan 1997). Fudge (1997) considered
concentrations < 150 mg/dL in avian species as life threatening; serum GLU levels fell below this threshold in only 5 Zn-dosed ducks, even though food consumption was reduced or had ceased an average of 6.5 days prior to death in the 29 Zn-dosed ducks, which succumbed to Zn intoxication (including those euthanized). Pectoral muscle atrophy was noted in all but 6 of the 29 Zn-dosed ducks which died or were euthanized prior to the end of the study, and visible subcutaneous and visceral fat stores were absent or minimal. Thus protein and fat catabolism may have contributed to the maintenance of blood GLU concentrations.

**Phosphorus**

Although Fairbrother et al. (1990) reported increased serum P concentrations in pre-laying and egg-laying mallards, mean values were higher in Zn- (only one of which was known to have laid any eggs) as compared with sham-dosed ducks in our study. Elevated P concentrations have also been associated with severe renal disease (Hochleithner 1994; Fudge 1997). In a similar study, Zn-dosed mallards had a higher mean plasma P concentration at Day 15 than Fe-dosed ducks (Levengood et al. 1997), and 67% percent of those Zn-dosed ducks that were examined histologically displayed mild to moderate necrosis of the epithelial cells of the renal tubules. Clinical signs and gross pathological changes were essentially identical to those observed in the current study. Mean P concentrations in both dosing groups were within the range of mean values for female mallards in the spring and summer months (Fairbrother et al. 1990), but higher than plasma values in sham- and W-Bi-Sn-dosed female mallards in the autumn (Ringelman et al. 1993).

**Total Protein**

Plasma or serum protein concentrations can be useful in diagnosing diseases of the
gastrointestinal tract, liver, or kidneys, and elevated levels may be indicative of inflammatory
disease, whereas low levels may be caused by starvation or malnutrition (Hochleithner 1994;
Fudge 1997). Although these conditions are characteristic of severely Zn-intoxicated mallards
(Levengood et al. 1997; this study), mean serum TP levels in Zn- and sham-dosed ducks were
similar to other studies (Fairbrother et al. 1990; Duncan 1997). Serum TP concentrations
increase during egg production (Fairbrother et al. 1990; Duncan 1997), and, presumably as a
consequence, were higher in sham-dosed ducks in our study.

Mean serum TP values were marginally higher ($P= 0.057$) in the PELLETS group ($\bar{x} =
4.4$ g/dL) than in the CORN/SOIL group ($\bar{x} = 3.9$ g/dL). Although the mean TP concentrations in
the PELLETS/SOIL and PELLETS groups were identical, we did not detect the difference
between the PELLETS/SOIL and CORN/SOIL cohorts at $\alpha = 0.05$ ($P = 0.10$). That TP levels
would be higher in treatment groups fed the pelletized ration is not surprising, given its higher
protein content relative to corn, however, we cannot explain the fact that the mean TP
concentration in the CORN group ($\bar{x} = 4.1$ g/dL) fell between the other 2 values.

**Uric Acid**

Elevated UA concentrations are considered diagnostic of renal dysfunction, and may also
result from dehydration and the liberation of nucleic acids due to severe tissue damage or
starvation (Hochleithner 1994; Fudge 1997). Renal pathology, pectoral muscle atrophy, visceral
gout, and cessation of feeding have been noted in Zn-intoxicated mallards (Levengood et al.
1997; this study). Fudge (1997) considered UA concentrations of 15-150 mg/dL as indicative of
renal disease. The mean value for, and concentrations in 11 individual, Zn-dosed ducks
exceeded this lower threshold. The mean serum UA concentration in sham-dosed ducks was

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lower than Fairbrother et al. (1990) reported for reproductive female mallards, and fell within the ranges of values documented by Duncan (1997) for sham-, Fe-, Bi-, and Pb-dosed mallards.

**Gross Pathology**

Eighteen of the 24 ducks dying before Day 30, and examined by the fourth author (GLF), evinced pectoral muscle atrophy; of these 6 had mild, 7 had moderate, and 5 had marked or severe loss of breast muscle mass. Fourteen of 23 (lesions were obscured by autolysis in one specimen) ducks had cecal lesions ranging from mild typhlitis to severe transmural necrosis. Lesions were ranked as mild in 2, moderate in 3, and marked or severe in 9 ducks. Ten ducks had mild to severe necrotizing intestinal enteritis, and this condition was associated with the cecal lesions. Of these 10 ducks, 2 were judged with mild, 3 with moderate, and 5 with severe lesions. Six ducks manifested pericardial and/or serosal mineralization, and this condition was severe in 2 of these animals. One duck had severe peritonitis with pericardial and coelomic effusions, another had severe coelomitis, and a third had severe pericardial effusion.

The incidence and severity of pectoral muscle atrophy in Zn-dosed ducks was similar across treatment groups. Typhlitis was more common and tended to be more severe in the corn-fed cohorts; 5 of 7 ducks in the CORN and 2 of 4 in the CORN/SOIL groups had marked to severe lesions. In contrast, only 1 of 5 ducks in the PELLETS and 1 of 8 in the PELLETS/SOIL groups had severe typhlitis. In 2 ducks (one each in the PELLETS/SOIL and CORN/SOIL groups) the inflammatory process had extended into the body cavity. Hepatic granulomas were observed in 3 ducks.

A similar pattern was observed for intestinal enteritis; 3 of 7 ducks in the CORN and 1 of 4 in the CORN/SOIL cohorts had severe lesions, whereas none of the ducks in the PELLETS and
1 of 8 in the PELLETS/SOIL groups had severe lesions involving the intestines.

Incidence and severity of lesions involving the pericardium or serosa were as follows:
CORN, 1; CORN/SOIL, 2 (1 severe); PELLETS, 0; PELLETS/SOIL, 3 (all marked or severe).

Each of the 5 ducks (1 CORN; 3 CORN/SOIL; 1 PELLETS) dying prior to Day 30, which were examined by the first author (JML), had lesions consistent with those identified above, including pectoral muscle atrophy, typhlitis (with associated intestinal enteritis in 2 cases), and pericardial and/or serosal mineralization or effusion (3 ducks). No significant lesions were noted in sham-dosed ducks, or in Zn-dosed ducks surviving to Day 30/31.

Gross lesions observed in Zn-dosed ducks in this study were consistent with those in ducks dosed with 6 No. 4 Zn shot and maintained on a corn diet (Levengood et al. 1997). Macroscopic lesions tended to be less severe in the groups fed the pelletized ration. Although the cecal and intestinal lesions play a role in the manifestation of clinical signs and reduced survival, they were not the sole determinant of mortality, which did not differ across the treatment groups.

CONCLUSIONS

Reduced survival, behavioral signs of intoxication, loss of body weight, organ hypertrophy or atrophy, reduction in PCVs, and gross pathological changes observed in this study for mallards dosed with 8 No. 4 Zn shot were consistent with the findings of Levengood et al. (1997), who dosed mallards with 6 No. 4 Zn shot of the same composition. In our study the overall mortality rate was lower, average survival time and the interval between dosing and the first mortality were longer, and fewer ducks displayed ataxia/paresis, as compared with female mallards in Levengood et al. (1997). However, mortality rates and the number of ducks exhibiting paralysis and incoordination did not differ significantly among dietary treatments in
the present study. Differences in survival and the manifestation of behavioral signs of intoxication between these studies may be attributable to the physiological state of the ducks, as determined by seasonality (i.e. spring vs. autumn).

Fewer ducks in the cohorts provided soil exhibited ataxia/paresis, though differences among groups were not significant. Total WBC counts were significantly higher in Zn-dosed ducks, and lower in sham-dosed ducks, which had soil available. One possible explanation is that this increase in WBC count resulted from the combined effects of Zn-mediated toxemia and reduced resistance to pathogens occurring naturally in the soil.

There were trends towards heavier organs and higher enzyme activities and metabolite concentrations in Zn-dosed ducks fed the commercial pelletized ration. Gross pathological changes involving the lower gut tended to be less severe in the pellet-fed groups, and to a lesser extent in those provided with floodplain soil. However, these lesions are not the sole determinant of mortality, as evidenced by similar mortality rates observed across the treatments.

Although the results of this study suggested that a more nutritionally-complete diet might provide some protection from Zn toxicosis, these effects were neither dramatic nor conclusive. The soil we used contained high levels of Ca, and P levels were higher in the duck pellet ration than in corn. Ca reduces the absorption and retention of Zn, particularly in the presence of P (Becker and Hoekstra 1971). Additionally, Ca and Fe have been demonstrated to inhibit the cytotoxicity of Zn (Borovansky and Riley 1989).

The effects of Ca in reducing Zn absorption and retention have been shown to decrease over time (Hoekstra 1964). This phenomenon may have been exacerbated by the relatively large dose of Zn (80.97 g Zn, or 1.0 mg Zn/g body weight, at initial dosing) and reduction/cessation
of feeding. This increasing Zn load and reduced food uptake may have also reduced the potential benefits of higher levels Fe, Cu, and protein, other dietary components for which antagonistic relationships with Zn have been demonstrated.

Zn levels in the diets provided ranged from 18 (soil) to 77 ppm (duck pellets). Although these concentrations added to the total Zn load, they constitute low dietary levels, far below the levels known to cause toxic effects in ducks and poultry. Because Zn is an essential element in animal nutrition, these low concentrations could have been beneficial to the ducks. The presence of these additional levels of dietary Zn, along with a small amount of Pb in the soil utilized, had no observed negative effects on sham-dosed ducks and produced no remarkable differences in toxic effects across Zn-dosed treatment groups.

The effects of diet in alleviating toxicosis were far less dramatic for Zn in our study than previously reported for Pb shot. In addition to the high dose of Zn, reduction in the effects of Ca on the absorption and retention of Zn over time (Hoekstra 1964), and reduced intake of Ca, P, and other Zn antagonists due to anorexia, the relative solubility and sorbing properties of Pb and Zn may have also played a role in the ability to alleviate toxicosis through changes in diet. Compared to Zn, Pb is less soluble, the adsorption of Pb by soils is greater over a wide pH range, and Pb readily precipitates with carbonates, phosphates, and sulfates and forms more stable compounds (de Haan and Zwerman 1978; Kerndorff and Schnitzer 1980; Kabat-Pendias and Pendias 1984; Elliott et al. 1986). Thus, given the low pH of the gizzard environment and a diet higher in components that affect the solubility and sorption of metals, more Pb may pass through the gut in the feces, thus reducing its availability for absorption, as compared with Zn.
ACKNOWLEDGMENTS

James W. Sergent and Brett A. Amdor, Illinois Natural History Survey (INHS) fed the ducks, cleaned their pens, and assisted with other phases of the study. Aaron P. Yetter, Michelle M. Georgi, Christopher S. Hine, and Bradley W. Zercher (all INHS) helped weigh, dose, and bleed the ducks. William R. Manuel, University of Illinois College of Veterinary Medicine (UICVM), retired, kindly provided his expertise in the collection of blood on Day 15. Linda K. Campbell, Kristi D. Caldwell, and Daniel J. Osterman (all INHS) assisted with data collection, recording, and entry. Karen L. Duncan, Dept. Veterinary Biosciences, UICVM, provided valuable advice regarding the collection and handling of blood for hematology and serum biochemistry determinations. Ivan G. Krapac, Geochemistry Section, Illinois State Geological Survey (ISGS), provided helpful discussions regarding the composition of soils. The particle size analysis of samples we collected were conducted in the Partial-Size Analysis Laboratory, ISGS, under the direction of Daniel J. Odomaitis. The shot, soil, duck pellet, and corn samples were prepared by Veronica Lasovsky, and the elemental analyses were conducted by Loretta M. Skowron, Office of Analytical and Water Treatment Services, Illinois State Water Survey. The Zn-dosed ducks were fluoroscoped by Heather N. Soder and Melinda K. Smith, under the direction of Richard L. Keen, Dept. of Veterinary Clinical Medicine, UICVM. With the exception of PCVs, hematological determinations were conducted by Karen Scrogum and Cher Godden, under the supervision of Kenneth R. Welle, All Creatures Animal Hospital, Urbana. Serum samples were analyzed by John W. Hoffman, under the direction of Walter E. Hoffmann, Dept. Veterinary Pathobiology, UICVM. Randall L. Peper, Office of Laboratory Animal Resources, UI, inspected Zn-intoxicated ducks and authorized euthanasia when necessary.

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Walter E. Hoffmann, UICVM, and Jeffrey D. Brawn, INHS/UI, kindly reviewed portions of an earlier draft of this report. The enthusiastic support of all those involved is gratefully acknowledged.

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Figure 1. Number, by diet, of 6-8 month-old game-farm mallards dosed with 8 No. 4 Zn shot exhibiting ataxia and/or paresis. CORN = whole kernal corn; PELLETS = duck pellet ration; SOIL = floodplain soil available.
Figure 2. Mean body weight, by diet, in 6-8 month-old game-farm mallards dosed with 8 No. 4 Zn shot. CORN = whole kernal corn; PELLETS = duck pellet ration; SOIL = floodplain soil available.
Figure 3. Mean organ weights, by diet, in 6-8 month-old game-farm mallards dosed with 8 No. 4 Zn shot. 
CORN = whole kernel corn; PELLETS = duck pellet ration; SOIL = floodplain soil available.
Figure 4. Mean PCV, by diet, in 6-8 month-old game-farm mallards dosed with 8 No. 4 Zn shot. CORN = whole kernal corn; PELLETS = duck pellet ration; SOIL = floodplain soil available.
Figure 5. Mean leukocyte counts at Day 15, by diet, in 6-8 month-old game-farm mallards dosed with 8 No. 4 Zn shot or sham-dosed. CORN = whole kernal corn; PELLETS = duck pellet ration; SOIL = floodplain soil available.
Figure 6. Mean lymphocyte counts at Day 15, by diet, in 6-8 month-old game-farm mallards dosed with 8 No. 4 Zn shot or sham-dosed. CORN = whole kernal corn; PELLETS = duck pellet ration; SOIL = floodplain soil available.
Figure 7. Serum enzyme activity and metabolite concentrations at Day 15, by diet, in 6-8 month-old game-farm mallards dosed with 8 No. 4 Zn shot. CORN = whole kernel corn; PELLETS = duck pellet ration; SOIL = floodplain soil available. See table 5 for units and definitions.
Table 1. Mean body weight ± SE of 6-8 month-old female game-farm mallards dosed with 8 No. 4 Zn shot or sham-dosed.a

<table>
<thead>
<tr>
<th>Dose</th>
<th>Mean Body Weight (Kg)</th>
<th>Day 0</th>
<th>Day 15b</th>
<th>Day 30c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0.99 ± 0.01</td>
<td>0.83 ± 0.02</td>
<td>0.89 ± 0.03 (n=43)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.03 ± 0.01</td>
<td>1.01 ± 0.02</td>
<td>1.03 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

a n= 60 unless otherwise specified
b ducks surviving ≤ 15 days
c ducks that survived > 15 days; all surviving ducks were euthanized on Day 30

Results of ANOVA testing:

Day 0
Dose $F_{1,112}= 5.0; P=0.03$

Day 15
Dose $F_{1,112}= 54.6; P<0.001$

Day 30
Dose $F_{1,95}= 26.1; P<0.001$
Diet $F_{3,93}= 3.2; P=0.03$
Table 2. Mean organ weights ± SE in 6-8 month-old female game-farm mallards dosed with 8 No. 4 Zn shot or sham-dosed.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Dose</th>
<th>Mean Organ Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn³</td>
<td>16.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>19.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Zn₃₀</td>
<td>18.5 ± 0.8 (n=31)</td>
</tr>
<tr>
<td></td>
<td>Zn₃₀</td>
<td>14.7 ± 0.7 (n=29)</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn³</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Zn₃₀</td>
<td>2.0 ± 0.1 (n=31)</td>
</tr>
<tr>
<td></td>
<td>Zn₃₀</td>
<td>1.4 ± 0.1 (n=29)</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn³</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Zn₃₀</td>
<td>6.0 ± 0.2 (n=31)</td>
</tr>
<tr>
<td></td>
<td>Zn₃₀</td>
<td>7.7 ± 0.3 (n=29)</td>
</tr>
<tr>
<td>Gonad</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn³</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Zn₃₀</td>
<td>3.1 ± 1.5 (n=31)</td>
</tr>
<tr>
<td></td>
<td>Zn₃₀</td>
<td>0.6 ± 0.1 (n=29)</td>
</tr>
<tr>
<td>Gizzard</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn³</td>
<td>29.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>25.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Zn₃₀</td>
<td>27.0 ± 0.8 (n=31)</td>
</tr>
<tr>
<td></td>
<td>Zn₃₀</td>
<td>14.4 ± 0.5 (n=29)</td>
</tr>
</tbody>
</table>
n= 60 for each group unless otherwise specified
*Zn-dosed ducks surviving < 30 days
'Zn-dosed ducks surviving 30 days; all surviving ducks were euthanized on Day 30
"Zn-dosed ducks surviving < 30 days

Results of ANOVA testing:

<table>
<thead>
<tr>
<th>Organ weight</th>
<th>Dose</th>
<th>Diet</th>
<th>F_{1,112}, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td>6.5, 0.01</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td></td>
<td>68.6, &lt;0.001</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td>7.6, 0.01</td>
</tr>
<tr>
<td>Gonad</td>
<td></td>
<td></td>
<td>21.7, &lt;0.001</td>
</tr>
<tr>
<td>Gizzard</td>
<td></td>
<td></td>
<td>26.2, &lt;0.001</td>
</tr>
</tbody>
</table>

For H_0: organ weight Zn_{30} = Zn_{<30}:

<table>
<thead>
<tr>
<th>Organ weight</th>
<th>t_{1,58}, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>4.0, &lt;0.001</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4.0, &lt;0.001</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.1, &lt;0.001</td>
</tr>
<tr>
<td>Gonad</td>
<td>2.9, 0.006</td>
</tr>
<tr>
<td>Gizzard</td>
<td>13.9, &lt;0.001</td>
</tr>
</tbody>
</table>
Table 3. Mean PCVs ± SE for 6-8 month-old female game-farm mallards dosed with 8 No. 4 Zn shot or sham-dosed.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Day 0</th>
<th>PCV (%)</th>
<th>Day 15</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>49.9 ± 0.5</td>
<td>38.7 ± 1.6</td>
<td>44.9 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n= 50)</td>
<td>(n= 50)</td>
<td>(n= 35)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>50.3 ± 0.6</td>
<td>48.2 ± 0.5</td>
<td>50.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n= 59)</td>
<td>(n= 59)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>n= 60 unless otherwise specified
<sup>b</sup>all surviving ducks were euthanized on Day 30

Results of ANOVA testing:

Hematocrit

<table>
<thead>
<tr>
<th>Day 15</th>
<th>Dose</th>
<th>$F_{1,101} = 35.3; P &lt; 0.001$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 30</td>
<td>Dose</td>
<td>$F_{1,87} = 10.2; P = 0.002$</td>
</tr>
</tbody>
</table>
Table 4. Mean total and differential leukocyte counts (per µL) at Day 15 in 6-8 month-old female game-farm mallards dosed with 8 No. 4 Zn shot or sham-dosed. Numbers in parentheses represent white blood cell types expressed as a percentage of total WBC count.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Total WBC</th>
<th>Heterophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>22,608 ± 2723</td>
<td>16,403 ± 2628</td>
<td>5,986 ± 2592</td>
<td>191.6 ± 51.6</td>
<td>17.1 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>(72.6)</td>
<td>(26.5)</td>
<td></td>
<td>(0.9)</td>
<td>(0.08)</td>
</tr>
<tr>
<td>0</td>
<td>14,592.9 ± 812</td>
<td>7,420 ± 578</td>
<td>6,932 ± 479</td>
<td>198.4 ± 30.1</td>
<td>13.7 ± 6.7</td>
</tr>
<tr>
<td></td>
<td>(50.9)</td>
<td>(47.5)</td>
<td></td>
<td>(1.4)</td>
<td>(0.10)</td>
</tr>
</tbody>
</table>

n= 45 for Zn-dosed, and n= 53 for sham-dosed, mallards

Results of ANOVA testing:

**Total WBC count**
- Dose: $F_{1,90} = 10.3; P=0.002$
- Dose x Diet: $F_{3,90} = 2.8; P=0.05$

**Heterophil count**
- Dose: $F_{1,90} = 21.8; P<0.001$

**Lymphocyte count**
- Diet: $F_{3,90} = 3.8; P=0.01$
- Dose x Diet: $F_{3,90} = 5.2; P=0.002$

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Table 5. Mean serum enzyme activities and metabolite concentrations ± SE at Day 15 in 6-8 month-old female game-farm mallards dosed with 8 No. 4 Zn shot or sham-dosed.*

<table>
<thead>
<tr>
<th>Dose</th>
<th>ALP(^{b})</th>
<th>AMYL</th>
<th>AST</th>
<th>CA</th>
<th>GLU</th>
<th>PHOS</th>
<th>TP</th>
<th>UA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>177.8 ± 24.5</td>
<td>3131.7 ± 259.8</td>
<td>225.8 ± 59.3</td>
<td>11.9 ± 0.6</td>
<td>190.0 ± 5.1</td>
<td>6.6 ± 0.6</td>
<td>3.5 ± 0.1</td>
<td>16.6 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>(n=43)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>312.9 ± 54.3</td>
<td>2510.5 ± 121.5</td>
<td>23.4 ± 1.6</td>
<td>15.3 ± 0.8</td>
<td>205.1 ± 3.7</td>
<td>4.8 ± 0.3</td>
<td>4.7 ± 0.1</td>
<td>4.6 ± 0.2</td>
</tr>
</tbody>
</table>

* n= 44 for Zn-dosed, and n= 59 for sham-dosed, unless otherwise specified

\(^{b}\) see below for definitions and units

Results of ANOVA testing:

**ALP** (alkaline phosphatase Units/L)

Dose \( F_{1,94} = 6.5; P=0.01 \)

**AMYL** (amylase U/L)

NS

**AST** (aspartate aminotransferase U/L)

Dose \( F_{1,95} = 37.6; P<0.001 \)

**CA** (calcium mg/dL)

Dose \( F_{1,95} = 12.9; P=0.001 \)

Diet \( F_{3,95} = 3.1; P=0.03 \)

**GLU** (glucose mg/dL)

Dose \( F_{1,94} = 6.8; P=0.01 \)

**PHOS** (phosphorus mg/dL)

Dose \( F_{1,95} = 12.6; P=0.001 \)

Diet \( F_{3,95} = 3.9; P=0.01 \)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source</th>
<th>Value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (total protein g/dL)</td>
<td>Dose</td>
<td>$F_{1,95} = 69.7$; $P&lt;0.001$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>$F_{3,95} = 2.9$; $P=0.04$</td>
<td></td>
</tr>
<tr>
<td>UA (uric acid mg/dL)</td>
<td>Dose</td>
<td>$F_{1,95} = 9.8$; $P=0.002$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>$F_{3,95} = 3.0$; $P=0.03$</td>
<td></td>
</tr>
</tbody>
</table>