INFLUENCE OF DIET ON THE HEMATOLOGY AND SERUM BIOCHEMISTRY OF ZINC-INTOXICATED MALLARDS

Authors: Levengood, Jeffrey M., Sanderson, Glen C., Anderson, William L., Foley, George L., Brown, Patrick W., et al.

Source: Journal of Wildlife Diseases, 36(1) : 111-123

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-36.1.111

Journal of Wildlife Diseases, 36(1), 2000, pp. 111–123

INFLUENCE OF DIET ON THE HEMATOLOGY AND SERUM BIOCHEMISTRY OF ZINC-INTOXICATED MALLARDS

Jeffrey M. Levengood,[1,4] Glen C. Sanderson,[1] William L. Anderson,[1] George L. Foley,[2,3] Patrick W. Brown,[1] and James W. Seets[1]

[1] Center for Wildlife Ecology, Illinois Natural History Survey, 607 E. Peabody Dr., Champaign Illinois 61820, USA
[2] Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, 2001 S. Lincoln Ave, Urbana, Illinois 61801, USA
[3] Present Address: Central Research Division, Pfizer, Inc., Eastern Point Rd., Bldg. 274, Groton, Connecticut 06340, USA
[4] Corresponding author (e-mail: jleven@mail.inhs.uiuc.edu)

ABSTRACT: Changes in hematological and serum biochemistry parameters in female zinc (Zn)-dosed farm-raised mallards (*Anas platyrhynchos*) fed four different diets were examined. Sixty ducks received an average dose of 0.97 g of Zn in the form of eight, 3.30-mm diameter shot pellets containing 98% Zn and 2% tin, and another 60 ducks were sham-dosed as controls. Fifteen ducks from each of the two dosing groups were assigned to one of four dietary treatments: corn only, corn with soil, commercial duck ration only, or commercial duck ration with soil. Shot-pellet dissolution rates ranged from 7 mg/Zn/day to 27 mg/Zn/day. Regardless of diet, the Zn dose resulted in mortality; incoordination; paralysis and anorexia; decreased body, liver, pancreas, gonad, and gizzard weight; increased kidney weight; and macroscopic lesions. Zn-dosed ducks had a lower mean erythrocyte packed cell volume (PCV), higher mean reticulocyte count, and a greater number of individuals with immature and/or abnormal erythrocytes, than did control mallards. Mean total leucocyte counts were higher in Zn-dosed ducks than in controls. Zn-dosed ducks that had soil available had higher leucocyte counts than those without soil. Zn-dosed ducks were characterized by a marked heterophilia and relative lymphopenia. In Zn-dosed ducks, the mean lymphocyte count was highest in those provided a commercial duck ration, and lowest in those fed corn. In control ducks, the mean lymphocyte count was highest in ducks fed corn, and lowest in those provided soil along with a commercial duck ration. Zn-dosed mallards had higher serum aspartate aminotransferase and amylase levels, and lower alkaline phosphatase activities than control ducks. Serum phosphorus and uric acid concentrations were higher, and calcium, glucose, and total protein levels lower, in Zn-dosed ducks than in control ducks. Diet did affect serum calcium, phosphorus, total protein, and uric acid concentrations. Differences in erythrocyte and leucocyte parameters, serum enzyme activities, and metabolite concentrations were associated with dose and diet effects. Diets high in protein and other organic matter and calcium and phosphorus did not prevent or substantially alleviate Zn toxicosis in farm-raised mallard ducks.

Key words: *Anas platyrhynchos*, clinical pathology, hematology, mallard, nontoxic shot, serum biochemistry, zinc.

INTRODUCTION

As a result of increased concern over the deposition of lead (Pb) shot pellets into wetlands by waterfowl hunters, a variety of alternative shot materials have been tested. Zinc (Zn)-coated Fe pellets are currently approved for waterfowl hunting in the United States, and pellets comprised primarily of Zn are currently, or were until recently, used for waterfowl hunting and other shooting applications in some European countries. Waterfowl also may be exposed to elevated Zn concentrations through ingestion of contaminated vegetation (Chupp and Dalke, 1964) and sediments (Chupp and Dalke, 1964; Beyer et al., 1997).

Birds are considered to be relatively resistant to Zn intoxication, however, intake at concentrations exceeding the physiological mechanisms to compensate can result in a variety of pathological effects (Eisler, 1993). Ducks have become intoxicated through the addition of Zn carbonate (Gasaway and Buss, 1972) or Zn sulfate (Kazacos and Van Vleet, 1989) to their diet or gavage of shot pellets comprised primarily of Zn (Grandy et al., 1968; Levengood et al., 1999). Mortality and weight loss in ducks dosed with eight, Zn-coated iron (Fe) or steel shot did not differ from

that of controls (Irby et al., 1967), and French et al. (1987) noted no abnormalities in ducks dosed with 5 or 10 nearly pure (99.9%) Zn shot. In contrast, dosing with eight and six shot pellets, respectively, comprised of 92% (Grandy et al., 1968) and 98% (Levengood et al., 1999) Zn produced toxicosis in mallards.

Comparisons among studies should consider differences in age, sex, dosing levels, season, length of studies, and diet. 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 (Sanderson and Bellrose, 1986). Dietary components such as calcium (Ca), phosphorus (P), and protein, along with the size and hardness of food items, are important in determining the susceptibility of ducks to lead poisoning through the ingestion of Pb shotshell pellets (Jordan, 1968; Sanderson and Bellrose, 1986). Evidence indicates that lead-poisoned ducks may select a diet that may help alleviate lead toxicosis (Jordan, 1968). Mayak et al. (1981) reported that kidney lesions in cadmium-dosed ducks were less severe in wood ducks fed a high protein diet, and protein level is considered perhaps the most critical dietary factor determining susceptibility of Pb poisoning (Sanderson and Bellrose, 1986). Diet might also influence Zn 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 calcium, 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; Eisler, 1993; Walsh et al., 1994). Addition of phytate to the diet of chickens may reduce the bioavailability/toxicity of Zn (Hempe and Savage, 1986; Lo et al., 1981; Thompson and Weber, 1981; Berg and Martinson, 1972), particularly in the presence of supplemental Ca and P (Bafundo et al., 1984).

The objective of this study was to examine the influence of four diets (whole kernel corn, and a commercial duck ration, each with or without the addition of soil) on the toxicity of Zn shot pellets to mallards. Changes in hematological and serum biochemistry parameters, considered in the context of other observed pathological changes, will be presented.

METHODS AND MATERIALS

Study design

The 30-day study was conducted with 120 female farm-raised mallards, which were 6 to 8 mo of age. After arrival at the Illinois Natural History Survey's duck-holding facility (Champaign, Illinois, USA; 40°05′N, 88°14′W), the ducks were weighed and randomly assigned to 1-m^2 pens and dosing group. Sixty ducks received an average dose of 0.97 g of Zn in the form of eight, 3.30-mm diameter (No. 4) shot pellets containing 98% Zn and 2% tin (Sn), and another 60 ducks were sham-dosed as controls. Fifteen ducks from each of the two dosing groups were assigned to one of four dietary treatments: corn only (CORN), corn with soil (CORN/SOIL), commercial duck ration only (RATION), or commercial duck ration with soil (RATION/SOIL). An animal-use protocol and our facilities were approved by the Laboratory Animal Care Advisory Committee of the University of Illinois (Champaign, Illinois, USA).

A commercial duck ration containing a minimum of 17.0% protein (Heinhold 17% Duck Finisher Pellet, Heinhold Feeds, Inc. Kouts, Indiana, USA) was provided *ad libitum* during a 20-day acclimatization period. On the date of dosing (2 June 1997), a 14% Duck Breeder Developer Pellet ration (Heinhold Feeds, Inc.) comprised of corn, soybean meal, ground oats, wheat middlings, alfalfa meal, meat and bone meal, and various vitamin and mineral supplements was provided to the RATION and RATION/SOIL groups. Guaranteed analysis was crude protein ≥14.0%, crude fat ≥2.0%, and crude fiber ≤8.0%. The mean concentrations (ppm dry weight [DW]) of selected elements in 12 composite pellet samples, as determined by Inductively Coupled Argon-Plasma Emission Spectroscopy (ICP) analysis, were: Ca, 5484; Cu, 11.6; Fe, 256; P, 6617; Pb, <Method Detection Limit (MDL) of 2.94; Sn, <MDL of 2.55; and Zn, 75.7. The CORN and CORN/SOIL groups were fed whole kernel corn obtained from the University of Illinois Department of Animal Sciences' feed mill. Crude protein levels in corn harvested in 1996 and ana-

lyzed by the Animal Sciences Laboratory averaged 9.5% (L. Bauer, pers. comm.). The mean concentrations (ppm DW) of selected elements in 12 composite corn samples, as determined by ICP analysis, were: Ca, 250; Cu, 2.2; Fe, 59.7; P, 2248; Pb, <MDL of 2.93; Sn, <MDL of 2.55; and Zn, 24.0.

Approximately 545.5 kg of soil were obtained from the Illinois River floodplain (Mason County, Illinois, USA; 40°17′N, 90°02′W). After tilling and grinding 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 (ppm DW) of selected elements in 10 soil samples, as determined by ICP analysis, were as follows: Ca, 4535; Cu, 7.0; Fe, 5946; P, 176; P, 13.4; Sn, <MDL of 2.28; and Zn, 17.7. Four soil samples were washed, sieved, and inspected both visually and radiologically; however, no foreign metallic objects were detected. Additionally, none was found in the gizzards of the ducks upon dissection. Soil was provided to the CORN/SOIL and RATION/SOIL groups in rubber feed tubs and was watered as necessary to maintain soil moisture.

Hematology

On day 0 (2 June 1997) the ducks were weighed, bled for packed cell volume (PCV) determination, and dosed by gavage with 0.97 g of Zn in the form of eight, 3.30-mm diameter shot pellets. Body weights were recorded and blood samples for PCVs were 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 of the ulnar vein 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 (Benton Dicksion Vacutainer Systems, Rutherford, New Jersey, USA) tubes for hematological analysis. An additional 1.5 to 2 ml of blood were transferred to untreated Vacutainer tubes and set aside for 1 hr before centrifugation and harvest of serum for determination of selected biochemistries.

Hematological parameters included PCV's, reticulocyte counts, erythrocyte morphology, total leucocyte count, leucocyte differential, leucocyte morphology, and thrombocyte counts. Microhematocrit tubes were spun for 5 min at 13,000-g force, and the PCV was determined using a Micro-Capillary Reader (Damon/IEC Division, Needham Heights, Massachusetts, USA). Erythrocyte and leucocyte morphologies were evaluated through microscopic examination of stained blood smears. The blood smears were stained using Dip Quick, a quick stain method (Jorgensen Laboratories, Inc., Loveland, Colorado, USA).

Differential leucocyte (WBC) counts were made by categorizing and counting the first 100 WBC's observed on a stained blood smear. The eosinophil Unopette system (Becton Dickinson and Co.) and a hemacytometer (American Optical Co., Buffalo, New York, USA) were used to obtain an absolute count of heterophils and 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 eight fields. Serum alkaline phosphatase (ALP), amylase (AMYL), and aspartate aminotransferase (AST) activity, and Ca, glucose (GLU), P, total protein (TP), and uric acid (UA) concentrations were determined using a Hitachi 705 Automated Chemistry Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, Indiana, USA).

Other parameters

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 dead ducks, and ascertain whether any ducks might be candidates for euthanasia. 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 retention of eight Zn shot pellets was confirmed by fluoroscopy on Days 7 and 8 (30 each day) at the University of Illinois College of Veterinary Medicine's Large Animal Radiology Laboratory (Urbana, Illinois, USA). All ducks (including control) were transported in poultry crates a distance of 1.6 km to the radiology lab. Control ducks were not fluoroscoped. Ducks retaining fewer than eight pellets were re-dosed to replace the missing pellets. The shot dissolution rate was determined by subtracting the weight of the shot collected at necropsy from the original weight of the shot dose, and dividing the resulting value by the number of days each duck survived. Analysis of chemical composition of the shot pellets was conducted at the Illinois State Water Survey's Analytical Laboratory (Champaign, Illinois, USA) with the use of Inductively Coupled Argon Emission Plasma Spectroscopy (ICP-AES). Ten randomly-selected pellets were composed

of an average of 98% Zn and 2% Sn; other elements were undetectable (<0.1% each).

All surviving ducks were weighed and blood was collected from the cutaneous ulnar vein on day 30. Subsequently, the ducks were euthanized by decapitation and necropsied on day 30 or 31. A board certified anatomic veterinary pathologist (4th author) necropsied 24 of 29 ducks dying before day 30; the additional five ducks, which succumbed prior to the end of the study, were examined by the first author. On day 30 the pathologist performed post-mortems on 10 randomly-selected control ducks. The remaining ducks were examined by project personnel on days 30 and 31.

Statistical methods

We examined variation in hematological variables and selected serum biochemistries by a randomized, 2 × 4 factorial, fixed effects ANOVA, with dose and diet as grouping factors; Tukey's HSD test was used for pairwise comparisons among treatments. Normality was assessed with the Kolmogorov-Smirnov Statistic with Lilliefors Significance Correction, the skewness statistic, and visual examination of data distributions. Parameters were $\log^e$ or arcsine (PCV) transformed to improve the distribution of data and reduce heterogeneity of variances among treatment groups. These procedures were available through SPSS statistical software (SPSS Inc., Chicago, Illinois, USA).

A probability level of $P \leq 0.05$ 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. These values were considered extremely low, and therefore, suspect. The PCV value (21%) for duck number 92 at Day 15 was also omitted; this duck was sham-dosed and had PCV's of 59% and 56% on Days 0 and 30, respectively.

RESULTS

Survival and clinical signs of intoxication

All control ducks survived to day 30/31, when the ducks were euthanized. Twenty-nine of 60 (48%) Zn-dosed ducks died or were euthanized prior to day 30. Mortality rates in Zn-dosed ducks did not differ among diets ($\chi^2_3 = 0.04$ to 0.68, $P \geq 0.41$). Survival in Zn-dosed ducks averaged 22.7 days, with the first mortality occurring on day 8. We did not detect a diet effect on the mean number of days survived within the Zn-dosed cohort ($F_{0.05(2),3,56} = 0.49$, $P = 0.69$). Forty-one of 60 (68%) Zn-dosed ducks displayed ataxia/paresis during the study. Within each diet group (corn and commercial duck ration), fewer ducks exhibited ataxia/paresis when soil was available; however, the number of ducks exhibiting such signs did not differ across groups ($\chi^2_3 = 0.87$, $0.75 < P < 0.90$). Zinc-dosed ducks became anorectic before showing overt signs of toxicosis. Other signs of toxicosis included dark or bright green feces, oral cavity pallor, diarrhea, foul-smelling excreta, passing of blood (three ducks), a drooping or tucked tail, clacking of the bill and other uncontrolled movements of the head, and evasive behavior indicative of diseased waterfowl.

Shot dissolution

Shot-pellet dissolution rates ranged from 16 mg/day in Zn-dosed ducks that retained all eight shot and died prior to day 30, to 25 mg/day in those that retained eight pellets and survived to day 30. Dissolution rates in individual ducks ranged from 7 mg/Zn/day to 27 mg/Zn/day. Small and disparate samples sizes did not allow a meaningful statistical comparison of pellet dissolution rates across treatment groups; however, there was a trend toward higher rates in the groups fed corn than those provided the commercial duck ration.

Body and organ weights

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 30, and a mean weight gain of 1% in control ducks. The livers, pancreases, gonads, and gizzards were reduced, and kidneys enlarged, in Zn-dosed ducks relative to controls.

Macroscopic lesions

Macroscopic lesions in those ducks that died or were euthanized prior to day 30 included pectoral muscle atrophy (79% of

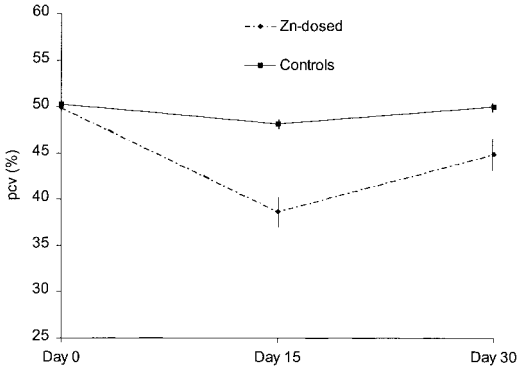

FIGURE 1. Mean packed cell volume in 6- to 8-mo-old game-farm mallards dosed with eight Zn shot pellets or sham-dosed.

those examined), cecal lesions ranging from mild typhlitis to severe transmural necrosis (68%), mild to severe necrotizing intestinal enteritis (41%), and pericardial and/or serosal mineralization (31%). One duck had severe peritonitis with pericardial and coelomic effusions, another had severe coelomitis, and a third had severe pericardial effusion. Hepatic granulomas were observed in three ducks. In addition, the linings of gizzards from Zn-dosed ducks were brittle and discolored. Whereas the incidence and severity of pectoral muscle atrophy in Zn-dosed ducks were similar across treatment groups, typhlitis and intestinal enteritis were more common and tended to be more severe in the ducks fed corn.

Packed cell volume (PCV)

Mean PCVs varied little over the course of the study in control ducks (Fig. 1). Mean PCV values decreased in surviving Zn-dosed ducks between days 0 and 15, before increasing between days 15 and 30. Although the higher mean PCV at day 30 to some degree reflected the loss (death) of severely-intoxicated ducks between days 15 and 30, some individuals showed an increase in PCV during this time. Mean PCV values were significantly lower in Zn-dosed, as compared with control ducks, at days 15 ($F_{0.05(2),1,101} = 35.3$, $P < 0.001$) and 30 ($F_{0.05(2),1,87} = 10.2$, $P = 0.002$) (Fig. 1). The lowest PCV value we documented was 13%, recorded for each of two Zn-dosed ducks just prior to euthanasia. We did not detect significant diet or dose × diet interaction effects on PCV's.

PCVs decreased by an average of 22% in Zn-dosed ducks and 4% in control ducks, between days 0 and 15. The change in PCV between days 0 and 15 ranged from −76% to +8% and −30% to +13% in Zn-dosed and control mallards, respectively. PCVs decreased by an average of 8.9% in Zn-dosed ducks and <1% in control ducks, between days 0 and 30. The change in PCV between days 0 and 30 ranged from −74% to +32% and −22% to +24% in Zn-dosed and control mallards, respectively.

Erythrocytes

The mean reticulocyte count at day 15 was significantly higher in Zn-dosed ($\bar{x}$ = 15.8/μl, SE = 1.3, n = 45) as compared with control ($\bar{x}$ = 7.7/μl, SE = 0.4, n = 53) ducks ($F_{0.05(2),1,90} = 38.5$, $P < 0.001$). Individual reticulocyte counts ranged from 3 to 35/μl and 4 to 18/μl in Zn-dosed and control mallards, respectively. We did not detect significant diet or dose × 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 control ducks. Basophilic or polychromatic prorubricytes were detected in the blood of 18 of 45 (40%) Zn-dosed and 5 of 53 (9%) control mallards. Poikilocytosis was not observed in control ducks but was noted in 11 Zn-dosed ducks, being judged as marked in five of these individuals. Binucleated erythrocytes were noted in one Zn-dosed individual.

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

TABLE 1. Mean total and differential leukocyte counts (per μl) at day 15 in 6- to 8-mo-old female game-farm mallards dosed with eight No. 4 Zn shot or sham-dosed.

Dose	Total WBC	Parameter			
		Heterophils	Lymphocytes	Monocytes	Eosinophils
Zn	22,608 ± 2,723[a]	16,403 ± 2,628 (72.6)[b]	5,986 ± 2,592 (26.5)	191.6 ± 51.6 (0.9)	17.1 ± 10.8 (0.08)
0	14,593 ± 812	7,420 ± 578 (50.9)	6,932 ± 479 (47.5)	198.4 ± 30.4 (1.4)	13.7 ± 6.7 (0.10)

[a] $n = 45$ for Zn-dosed and $n = 53$ for sham-dosed mallards.
[b] Numbers in parentheses represent white blood cell types expressed as a percentage of total WBC count.

Leucocytes

The mean total leukocyte (WBC) count was significantly higher ($F_{0.05(2),1,90} = 10.3$, $P = 0.002$) in Zn-dosed as compared with control ducks (Table 1). We also detected a marginally significant interaction effect ($F_{0.05(2),3,90} = 2.8$, $P = 0.05$); WBC counts in Zn-dosed ducks were higher for treatment groups that had soil available, regardless of diet (Fig. 2). WBC counts in control 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-dosed and control ducks, respectively.

Zinc-dosed ducks had a larger proportion of heterophils and smaller proportion of lymphocytes, relative to control ducks (Table 1). Zn-dosed ducks also had slightly smaller proportions of monocytes and eosinophils than control ducks. The mean heterophil count was significantly greater in Zn-dosed as compared with control ducks ($F_{0.05(2),1,90} = 21.8$, $P < 0.001$). Individual heterophil counts ranged from 3,907 to 93,403/μl in Zn-dosed ducks and 1,971 to 23,056/μl in control ducks. We did not detect statistically significant diet or diet × dose interaction effects on mean heterophil counts. Toxic changes were detected in the heterophils of 11 of 45 (24%) Zn-dosed ducks, compared with only one of 53 (2%) control mallards. The severity of these changes was ranked as +1 in two, +2 in seven, and +3 in two Zn-dosed mallards.

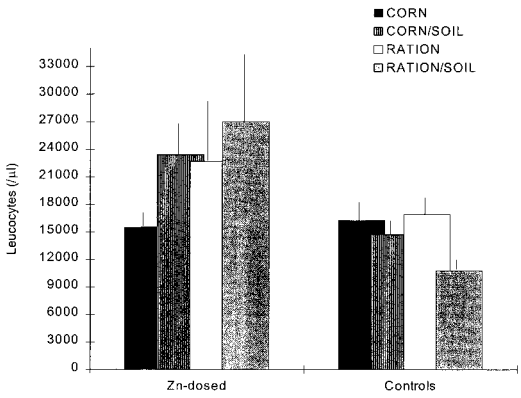

FIGURE 2. Mean leukocyte counts at day 15, by diet, in 6- to 8-mo-old game-farm mallards dosed with eight Zn shot pellets or sham-dosed. CORN = whole kernel corn; RATION = commercial duck ration; SOIL = floodplain soil available.

The mean lymphocyte count was lower in Zn-dosed than in control ducks (Table 1); however, this difference was not statistically 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 control ducks. We detected a diet effect on lymphocyte counts ($F_{0.05(2),3,90} = 3.8$, $P = 0.01$); post hoc testing revealed that the mean lymphocyte count was lower in the RATION/SOIL group than in the CORN or RATION groups (Fig. 3). We also detected a significant dose × diet interaction effect on lymphocyte counts ($F_{0.05(2),3,90} = 5.2$, $P = 0.002$). Reactive lymphocytes were observed in the blood of 14 of 45 (31%) Zn-dosed and 11 of 53 (21%) control ducks.

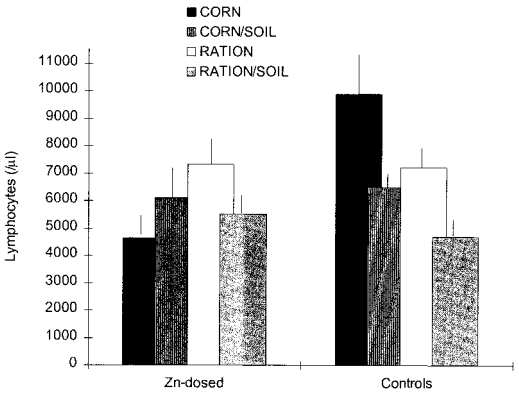

FIGURE 3. Mean lymphocyte counts at day 15, by diet, in 6- to 8-mo-old game-farm mallards dosed with eight Zn shot pellets or sham-dosed. CORN = whole kernel corn; RATION = commercial duck ration; SOIL = floodplain soil available.

Serum enzymes and metabolites

Regardless of diet, Zn-dosed ducks had greater mean serum AST activity ($F_{0.05(2),1,95} = 37.6$, $P < 0.001$) and P ($F_{0.05(2),1,95} = 12.6$, $P = 0.001$) and UA ($F_{0.05(2),1,95} = 9.8$, $P = 0.002$) concentrations, and lower ALP activity ($F_{0.05(2),1,94} = 6.5$, $P = 0.01$) and Ca ($F_{0.05(2),1,95} = 12.9$, $P = 0.001$), GLU ($F_{0.05(2),1,94} = 6.8$, $P = 0.01$), and TP ($F_{0.05(2),1,95} = 69.7$, $P < 0.001$) concentrations, than control ducks (Table 2). Zn-dosed ducks experienced nearly 10- and 4-fold increases in mean AST activity and uric acid concentration, respectively, as compared with control ducks. Although AMYL activities were 25% higher in Zn-dosed mallards, this difference between dosing groups was not statistically significant ($P = 0.10$).

Diet effects were detected for mean Ca ($F_{0.05(2),3,95} = 3.1$, $P = 0.03$), P ($F_{0.05(2),3,95} = 3.9$, $P = 0.01$), TP ($F_{0.05(2),3,95} = 2.9$, $P = 0.04$), and UA ($F_{0.05(2),3,95} = 3.0$, $P = 0.03$) concentrations (Fig. 4). Post-hoc testing revealed that mean serum Ca and UA levels were higher in the RATION/SOIL group than in the CORN group. Phosphorus concentrations in the RATION/SOIL group averaged higher than in each of the other treatment groups. Mean TP concentrations were marginally greater ($P = 0.057$) in the RATION group,

TABLE 2. Mean serum enzyme activities and metabolite concentrations ± SE at day 15 in 6- to 8-mo-old female game-farm mallards dosed with eight No. 4 Zn shot or sham-dosed.

Dose	Serum parameter							
	ALP[a]	AMYL	AST	Ca	GLU	P	TP	UA
Zn	168.0 ± 24.2[b]	3,131.7 ± 259.8	225.8 ± 59.3	11.9 ± 0.6	185.8 ± 6.5	6.6 ± 0.6	3.5 ± 0.1	16.6 ± 4.0
0	312.9 ± 54.3	2,510.5 ± 121.5	23.4 ± 1.6	15.3 ± 0.8	205.1 ± 3.7	4.8 ± 0.3	4.7 ± 0.1	4.6 ± 0.2

[a] Definitions and units are as follows: alkaline phosphatase (ALP), Units/L (U/L); amylase (AMYL), U/L; aspartate aminotransferase (AST), U/L; calcium (Ca), mg/dl; glucose (GLU), mg/dl; phosphorus (P), mg/dl; total protein (TP), g/dl; uric acid (UA), mg/dl.
[b] $n = 44$ for Zn-dosed and $n = 59$ for sham-dosed ducks.

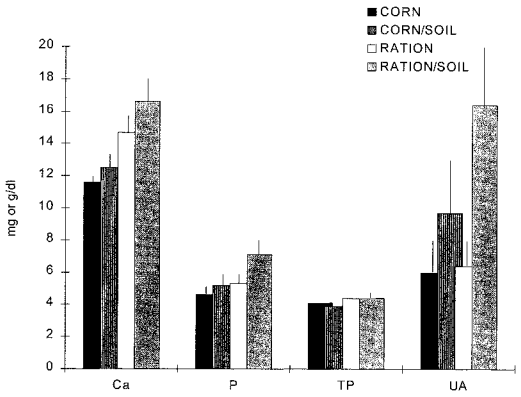

FIGURE 4. Serum metabolite concentrations at day 15, by diet, in 6- to 8-mo-old game-farm mallards dosed with eight Zn shot pellets or sham-dosed. Ca = calcium (mg/dl), P = phosphorus (mg/dl), TP = total protein (g/dl), UA = uric acid (mg/dl). CORN = whole kernel corn; RATION = commercial duck ration; SOIL = floodplain soil available.

as compared with the CORN/SOIL cohort. No statistically significant interaction effects were detected for serum chemistries.

DISCUSSION

Anemia is common in Zn-intoxicated vertebrates (Eisler, 1993). High levels of Zn can lead to faulty hematopoiesis and shortened erythrocyte life span caused by alterations in Cu and Fe absorption and metabolism (Walsh et al., 1994; Underwood, 1971; Pimentel et al., 1992). PCV values in undosed, farm-raised mallards average 45%–46% (Ringelman et al., 1993; Duncan, 1997; Levengood et al., 1999; Sanderson et al., 1997a, b). 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; however they were below 35% in only 4 of 35 (11%) Zn-dosed ducks at day 30 or at the time of euthanasia. We also detected a higher mean reticulocyte count, increased polychromasia, and incidence of immature erythrocytes in Zn-dosed ducks. Increases in reticulocyte counts, immature erythrocytes, and the degree of polychromasia are regarded as indicators of an erythrocyte regenerative response (Campbell, 1994; Fudge, 1997). Thus, our results suggest that surviving Zn-dosed ducks were able to respond successfully to the Zn-induced anemia.

The average total WBC count in Zn-dosed ducks (22,608/μl) was greater than the largest mean value documented by Duncan (1997) for Pb-dosed mallards (19,394/μl), and our maximum value (99,365/μl) exceeded the 51,200/μl reported by Hemm and Carleton (1967) in their review of duck hematology. Higher WBC counts in Zn-dosed ducks which had soil available may have resulted from the combined effects of Zn-mediated toxemia and reduced resistance to pathogens occurring naturally in the soil. Heterophilias can be indicative of inflammatory diseases as well as toxicities, and are often the cause of leucocytosis, the magnitude of which often reflects the extent of the inflammatory response (Campbell, 1994). The slight heterophilia observed in control ducks was consistent with the mild inflammatory changes considered normal for farm-raised mallards (Sanderson et al., 1997b). Marked heterophilia in Zn-dosed ducks was consistent with the gastrointestinal lesions observed in this study, as well as gross and microscopic lesions reported by Levengood et al. (1999) for mallards dosed with six Zn shot pellets of the same size and composition.

The mean ALP activity in both Zn-dosed and control ducks at Day 15 of our study was greater than the range of means provided by Fairbrother et al. (1990) for hen mallards at various reproductive stages. Gonadal development was arrested in Zn-dosed ducks, and only one was known to have laid eggs during our 30-day study, whereas all of the control ducks laid ≥1 egg. ALP levels are also known to increase during egg-laying (Fairbrother et al., 1990), and greater activity levels in control than in Zn-dosed ducks likely reflected the reproductive status of the respective cohorts.

Pectoral muscle atrophy, degeneration and necrosis of cardiac myofibers, and hepatocellular atrophy and apoptosis have been noted in Zn-dosed ducks (Levengood et al., 1999). Increased AST activity may reflect liver or muscle tissue damage; activities of 300–15,000 Units/L (U/L) are indicative of liver, muscle, or heart damage in avian species (Fudge, 1997). Hochleithner (1994) considered levels >230 U/L in avian species as abnormal. In the present study the mean value approached, and activity in 12 Zn-dosed individuals exceeded, the 230 U/L threshold.

Mean serum AMYL activities in Zn-dosed and control mallards were within the range reported by Fairbrother et al. (1990) for mallards from the pre-egg laying through postreproductive stages. Although Fairbrother et al. (1990) found AMYL activity levels to be greatest during egg laying, the mean amylase value was greater in Zn-dosed than in control ducks in our study. AMYL catalyzes the hydrolysis of complex carbohydrates, and the highest activities are found in the pancreas and duodenum (Kramer, 1989). Thus, elevated plasma or serum AMYL activity associated with pathological conditions is considered indicative of pancreatitis or enteritis (Hochleithner, 1994). Levengood et al. (1999) found Zn-intoxicated ducks had pancreatic apoptosis, typhlitis, and enteritis involving the small and large intestines; clinical signs and gross pathological changes in Zn-intoxicated ducks observed in their study were essentially identical to those observed in this study.

Serum Ca concentrations increase during egg production (Fairbrother et al., 1990), and mean serum Ca concentrations in our study were higher in control ducks, which, as a group, laid eggs throughout the 30-day study. Mean serum Ca levels in the present study were within the range of means reported by Fairbrother et al. (1990) and Duncan (1997). Mean Ca concentrations were approximately 20 times greater in the commercial duck ration than in corn, and concentrations in the soil provided to the ducks were higher than in the commercial duck ration. Thus, serum Ca concentrations reflected the Ca content of the respective diets.

Although serum GLU concentrations were higher in control than in Zn-dosed mallards, mean values in both groups were consistent with values reported in other studies (e.g., Fairbrother et al., 1990). Fudge (1997) considered concentrations <150 mg/dl in avian species as life threatening. In the present study, serum GLU levels fell below this threshold in only five Zn-dosed ducks, although food consumption was reduced or had ceased an average of 6.5 days prior to death in the 29 Zn-dosed ducks that succumbed to Zn intoxication (including those euthanized). Pectoral muscle atrophy was notable in the majority of the 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 in spite of decreased food consumption.

Fairbrother et al. (1990) reported increased serum P concentrations in pre-laying and egg-laying mallards. Only one Zn-dosed duck laid any eggs, although mean serum P concentrations were higher in the Zn-dosed ducks, when compared to control ducks. Elevated P concentrations have also been associated with severe renal disease (Hochleithner, 1994). In a similar study, Zn-dosed mallards had a higher mean plasma P concentration 15 days after dosing when compared to Fe-dosed ducks (Levengood et al., 1999). Sixty-seven percent of those Zn-dosed ducks that were examined histologically in that study 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. These results suggest that high concentrations of Zn increase serum P levels by damaging renal tissue.

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 depressed concentrations may be caused by starvation or malnutrition (Hochleithner, 1994). Although these conditions are characteristic of severely Zn-intoxicated mallards (Levengood et al., 1999), mean serum TP levels in Zn-dosed and control ducks were similar to other studies (e.g., Fairbrother et al., 1990), and thus did not reflect Zn toxicosis. Serum TP concentrations increase during egg production (Fairbrother et al., 1990), and presumably as a consequence, were higher in control ducks in our study.

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). Renal pathology, pectoral muscle atrophy, visceral gout, and cessation of feeding have been noted in Zn-intoxicated mallards in this study and others (e.g., Levengood et al., 1999). Fudge (1997) considered UA concentrations of 15–150 mg/dl as indicative of renal disease. The UA concentration in 11 Zn-dosed ducks exceeded this lower threshold. The mean serum UA concentration in control ducks was 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.

Our results indicated that diets high in protein and other organic materials and in Ca, P, and Fe provided little protection from Zn toxicosis. The commercial duck ration we fed our ducks contained greater protein concentrations than corn, and the ration/soil diet contained the greatest amount of protein of the four diet regimes. The gross pathological lesions that were observed involving the lower gut were less severe in ducks fed the commercial duck ration, and fewer ducks that were provided with soil exhibited paralysis and incoordination. It is unknown whether these differences resulted from the elevated protein levels, higher concentrations of essential elements in ration and soil-fed ducks, and/or greater dissolution of Zn pellets in corn-fed groups.

The commercial ration and soil we used contained high levels of Ca, and Ca and P levels were higher in the duck pellet ration than in corn. In fact, serum Ca and P concentrations were highest in the RATION/SOIL group. Ca reduces the absorption and retention of Zn, particularly in the presence of P (Becker and Hoekstra, 1971), and Bafundo et al. (1984) found that Ca in the presence of plant protein reduced the amount of Zn present in the tissues of young chicks. Ca and Fe have been demonstrated to inhibit the cytotoxicity of Zn (Borovansky and Riley, 1989). The effect of Ca in reducing Zn absorption and retention has been shown to decrease over time (Hoekstra, 1964), and this reduction in the ability of Ca to antagonize Zn may have been exacerbated by the relatively large dose of Zn and reduction/cessation of feeding. This increasing Zn load and reduced food uptake may have also reduced the potential benefits of higher levels of Fe, Cu, and protein, other dietary components for which antagonistic relationships with Zn have been demonstrated. In addition, the relative solubility and sorbing properties of Pb and Zn may have also played a role. 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.

Diets higher in protein, Ca, and P were not effective in alleviating toxicosis, as has been previously reported for Pb shot, even though the daily and total dissolution rates were similar to those of Pb-dosed ducks in other studies (Sanderson et al., 1992, 1997b). Regardless of diet, elemental Zn produced mortality, macroscopic lesions, behavioral changes, and changes in hematology and serum enzymes and metabolites. Although we did not measure tissue Zn retention in this study, liver, kidney, and pancreas Zn concentrations were greatly elevated in a previous study producing similar clinical and pathological results (Levengood et al., 1999).

It has been suggested that ducks may select diets to alleviate the symptoms of Pb poisoning (Jordan, 1968; Havera and Anderson, 1999). However, the results of the present study indicated that, where toxic concentrations of Zn are available to ducks, diet cannot be expected to allay the effects of Zn intoxication. There is increasing concern about anthropogenic sources of Zn, and highly elevated Zn concentrations have been documented in areas frequented by ducks (e.g., Coeur D'Alene River, Chupp and Dalke, 1964; Illinois River, Cahill and Steele, 1986; Cahill et al., 1995). Future research efforts should address the bioavailability and effects of environmental concentrations of Zn to ducks and other waterbirds.

A suite of hematological and biochemical parameters, including PCV, reticulocyte counts, RBC morphology, total WBC counts and differentials, and serum enzymes and metabolites, were examined in this study. These parameters could be utilized as biomarkers of effect after exposure of waterfowl to Zn. Overt signs of Zn intoxication (e.g., ataxia/paresis, gross pathology) observed in the present study were identical to those associated with Zn concentrations of 62 to 609 ppm in the kidneys, 142 to 597 ppm in the liver, and 752 to 3,844 ppm in the pancreas of farm-raised mallards in a similar study (Levengood et al., 1999). In the absence of clinical disease, or in concert with other signs of intoxication, these blood parameters could be used to form a presumptive diagnosis of subclinical or clinical Zn poisoning when waterfowl are known, or suspected, to be exposed to elevated concentrations of Zn.

ACKNOWLEDGMENTS

The assistance and expertise of J. Sergent, B. Amdor, L. Campbell, K. Caldwell, D. Osterman, A. Yetter, M. Georgi, C. Hine, B. Zercher, J. Brawn, W. Hoffmann, R. Peper, R. Keen, W. Manuel, K. Duncan, H. Soder, M. Smith, J. Hoffman, K. Scrogum, C. Godden, K. Welle, I. Krapac, D. Adomaitis, V. Lasovsky, L. Skowron, and two anonymous reviewers are most gratefully acknowledged.

LITERATURE CITED

BAFUNDO, K. W., D. H. BAKER, AND P. R. FITZGERALD. 1984. Zinc utilization in the chick as influenced by dietary concentrations of calcium and phytate and by *Eimeria acervulina* infection. Poultry Science 63: 2430–2437.

BECKER, W. M., AND W. G. HOEKSTRA. 1971. The intestinal absorption of zinc. *In* Intestinal absorption of metal ions, trace elements, and radionuclides, S. C. Skoryna and D. Waldron-Edward (eds.). Pergamon Press, Oxford, UK, pp. 229–256.

BERG, L. R., AND R. D. MARTINSON. 1972. Effect of diet composition on the toxicity of zinc for the chick. Poultry Science 51: 1690–1695.

BEYER, W. N., L. J. BLUS, C. J. HENNY, AND D. AUDET. 1997. The role of sediment ingestion in exposing wood ducks to lead. Ecotoxicology 6: 181–186.

BOROVANSKY, J., AND P. A. RILEY. 1989. Cytotoxicity of zinc in vitro. Chemical-Biological Interactions 69: 279–291.

CAMPBELL, T. W. 1988. Avian hematology and cytology. Iowa State University Press, Ames, Iowa, 101 pp.

———. 1994. Hematology. *In* Avian medicine: Principles and application, B. W. Ritchie, G. J. Harrison, and L. R. Harrison (eds.). Wingers Publ., Lake Worth, Florida, pp. 76–199.

CAHILL, R. A., J. D. STEELE, AND R. H. HENDERSON. 1995. Chemical characterization of sediments collected at DePue Lake sediment disposal area. Illinois State Geological Survey Champaign, Illinois, 9 pp.

———, AND ———. 1986. Inorganic composition and sedimentation rates of backwater lakes associated with the Illinois River. Environmental Geology Notes 115, Illinois State Geological Survey, Champaign, Illinois, 61 pp.

CHUPP, N. R., AND P. D. DALKE. 1964. Waterfowl mortality in the Couer D'Alene River Valley, Idaho. The Journal of Wildlife Management 28: 692–702.

DE HAAN, F. A. M., AND P. J. ZWERMAN. 1978. Pollution of soil. *In* Soil chemistry: A. Basic elements, G. H. Bolt and M. G. M. Bruggenwert (eds.). Elsevier Publ., Amsterdam, Netherlands, pp. 192–271.

DUNCAN, K. L. 1997. The effects of oral dosing of bismuth alloy, steel, or lead shot on the reproduction, biochemistry, hematology, and tissue residues of game-farm mallards. M.S. Thesis, University of Illinois, Urbana-Champaign, Illinois, 135 pp.

EISLER, R. 1993. Zinc hazards to fish, wildlife and invertebrates: A synoptic review. U.S. Fish Wildlife Service Biological Report 10, Contaminant Hazard Reviews Report 26, Washington, D.C., 106 pp.

ELLIOTT, H. A., M. R. LIBERATI, AND C. P. HUANG. 1986. Competitive adsorption of heavy metals by soil. Journal of Environmental Quality 15: 214–219.

FAIRBROTHER, A., M. A. CRAIG, K. WALKER, AND D. O'LOUGHLIN. 1990. Changes in mallard (*Anas platyrhynchos*) serum chemistry due to age, sex, and reproductive condition. Journal of Wildlife Diseases 26: 67–77.

FRENCH, M. C., C. W. HAINES, AND J. COOPER. 1987. Investigation into the effects of ingestion of zinc shot by mallard ducks (*Anas platyrhynchos*). Environmental Pollution 47: 305–314.

FUDGE, A. M. 1997. Avian clinical pathology-hematology and chemistry. *In* Avian medicine and surgery, R. B. Altman, S. L. Clubb, G. M. Dorrestein, and K. Quesenberry (eds.). W. B. Saunders Co., Philadelphia, Pennsylvania, pp. 142–157.

GASAWAY, W. C., AND I. O. BUSS. 1972. Zinc toxicity in the mallard duck. The Journal of Wildlife Management 36: 1107–1117.

GRANDY, J. W., L. N. LOCKE, AND G. E. BAGLEY. 1968. Relative toxicity of lead and five proposed shot types to pen-reared mallards. The Journal of Wildlife Management 32: 483–488.

HAVERA, S. P., AND W. L. ANDERSON. 1999. Food contents of mallard gizzards with and without ingested lead or steel shot. Transactions of the Illinois State Academy of Science 92: 89–94.

HEMM, R., AND W. W. CARLTON. 1967. Review of duck hematology. Poultry Science 46: 956–962.

HEMPE, J. M., AND J. E. SAVAGE. 1986. Effect of soybean flour on the bioavailability of zinc from zinc carbonate. Poultry Science 65: 58.

HOCHLEITHNER, M. 1994. Biochemistries. *In* Avian medicine: Principles and application. B. W. Ritchie, G. J. Harrison, and L. R. Harrison (eds.). Wingers Publ., Lake Worth, Florida, pp. 223–245.

HOEKSTRA, W. G. 1964. Recent observations on mineral interrelationships. Proceedings of the Federation of American Societies for Experimental Biology 23: 1068–1076.

IRBY, H. D., L. N. LOCKE, AND G. E. BAGLEY. 1967. Relative toxicity of lead and selected substitute shot types to game-farm mallards. The Journal of Wildlife Management 31: 253–257.

JORDAN, J. S. 1968. Influence of diet in lead poisoning in waterfowl. Transactions 25th Northeast Fish and Wildlife Conference 25: 143–170.

KABATA-PENDIAS, A., AND H. PENDIAS. 1984. Trace elements in soils and plant. CRC Press, Boca Raton, Florida, 315 pp.

KAZACOS, E. A., AND J. F. VAN VLEET. 1989. Sequential ultrastructural changes of the pancreas in zinc toxicosis in ducklings. American Journal of Pathology 134: 581–595.

KERNDORFF, H., AND M. SCHNITZER. 1980. Sorption of metals on humic acid. Geochimica et Cosmochimica Acta 44: 1701–1708.

KRAMER, J. W. 1989. Clinical enzymology. *In* Clinical biochemistry of domestic animals. J. J. Kaneko (ed.). Academic Press; Inc., San Diego, California, pp. 338–363.

LEVENGOOD, J. M., G. C. SANDERSON, W. L. ANDERSON, G. L. FOLEY, L. M. SKOWRON, P. W. BROWN, AND J. W. SEETS. 1999. Acute toxicity of zinc shot to game-farm mallards. Illinois Natural History Survey Bulletin 36: 1–36.

LO, G. S., S. L. SETTLE, F. H. STEINKE, AND D. T. HOPKINS. 1981. Effect of phytate : zinc molar ratio and isolated soybean protein on zinc bioavailability. Journal of Nutrition 111: 2223–2235.

MAYAK, L. A., P. B. BUSH, O. J. FLETCHER, R. K. PAGE, AND T. T. FENDLEY. 1981. Tissue residues of dietary cadmium in wood ducks. Archives of Environmental Contamination and Toxicology 10: 637–645.

PIMENTAL, J. L., J. L. GREGER, M. E. COOK, AND J. L. STAHL. 1992. Iron metabolism in chicks fed various levels of zinc and copper. Journal of Nutritional Biochemistry 3: 140–145.

RINGELMAN, J. K., M. W. MILLER, AND W. F. ANDELT. 1993. Effects of ingested tungsten-bismuth-tin shot on captive mallards. The Journal of Wildlife Management 57: 725–732.

SANDERSON, G. C., AND F. C. BELLROSE. 1986. A review of the problem of lead poisoning in waterfowl. Illinois Natural History Survey Special Publication No. 4, Champaign, Illinois, 34 pp.

———, S. G. WOOD, G. L. FOLEY, AND J. D. BRAWN. 1992. Toxicity of bismuth shot compared with lead and steel shot in game-farm mallards. Transactions 57th North American Wildlife and Natural Resources Conference 57: 526–540.

———, W. L. ANDERSON, G. L. FOLEY, L. M. SKOWRON, J. D. BRAWN, AND J. W. SEETS. 1997a. Acute toxicity of ingested bismuth alloy shot in game-farm mallards. Illinois Natural History Survey Bulletin 35: 185–216.

———, W. L. ANDERSON, G. L. FOLEY, K. L. DUNCAN, L. M. SKOWRON, J. D. BRAWN, AND J. W. SEETS. 1997b. Toxicity of ingested bismuth alloy shot in game farm mallards: Chronic health effects and effects on reproduction. Illinois Natural History Survey Bulletin 35: 217–252.

THOMPSON, S. A., AND C. W. WEBER. 1981. Effect of dietary fiber sources on tissue mineral levels in chicks. Poultry Science 60: 840–845.

UNDERWOOD, E. J. 1971. Trace elements in human and animal nutrition. Academic Press, New York, New York, 543 pp.

WALSH, C. T., H. H. SANDSTEAD, A. S. PRASAD, P. M. NEWBERNE, AND P. J. FRAKER. 1994. Zinc: Health effects and research priorities for the 1990s. Environmental Health Perspectives 102: 5–46.

Received for publication 26 March 1999.