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REPRODUCTION IN EASTERN SCREECH-OWLS FED SELENIUM

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Abstract: Raptors are occasionally exposed to excessive selenium from contaminated prey, but the effe of this exposure on reproduction are unknown. Therefore, we fed captive eastern screech-owls (*Otus as* diets containing 0, 4.4, or 13.2 ppm (wet wt) added selenium in the form of seleno-DL-methionine. Ad mass at sacrifice and reproductive success of birds receiving 13.2 ppm selenium were depressed ($P < 0.0$ relative to controls. Parents given 4.4 ppm selenium produced no malformed nestlings, but femur lengths young were shorter ($P = 0.015$) than those of controls. Liver biochemistries indicative of oxidative stress we affected ($P < 0.05$) in 5-day-old nestlings from parents fed 4.4 ppm selenium and included a 19% increa in glutathione peroxidase activity, a 43% increase in the ratio of oxidized glutathione (GSSG) to reduc glutathione (GSH), and a 17% increase in lipid peroxidation. Based on reproductive effects relative to dieta exposure, sensitivity of eastern screech-owls to selenium was similar to that of black-crowned night-here (*Nycticorax nycticorax*) but less than that of mallards (*Anas platyrhynchos*).

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Excessive selenium exposure has been associated with irrigation drainwater in arid wetlands of the western United States (Lemly et al. 1993), but also may result from fly ash from coal-fired power plants (King et al. 1994), and mining of phosphates and metal ores. Aquatic birds may acquire toxic levels of selenium, a common trace element, through contaminated aquatic food (Ohlendorf et al. 1986, Presser and Ohlendorf 1987, Ohlendorf 1989). For example, high rates of embryonic mortality, abnormalities of young, and reductions in reproductive success occurred in nesting aquatic birds at Kesterson Reservoir (Ohlendorf et al. 1986, 1988*a*; Williams et al. 1989) and adjacent areas (Ohlendorf and Skorupa 1989, Skorupa and Ohlendorf 1991) in the Central Valley of California.

High concentrations of selenium in prey may be a threat to predatory birds in areas of contamination (Clark 1987). At Kesterson, selenium concentrations in small mammals (Clark 1987) and in breast tissue of ducks and American coots (*Fulica americana*; Ohlendorf et al. 1986) were similar to those that caused adverse effects on reproduction in mallards (Heinz et al. 1987, 1989). However, a similar concentration (10 ppm, dry wt) did not affect hatching success or cause developmental malformations in blac crowned night-herons (Smith et al. 1988). Co centrations of selenium in snakes and frogs fro Kesterson were high enough to warrant conce regarding effects on predators (Ohlendorf et 1988*b*).

The primary objective of this study was determine if dietary selenium levels similar and above those found in raptor prey items fro selenium-contaminated environments would a fect reproduction of captive eastern screech-ow We also examined changes in mass of adul selenium concentrations in livers of adults an eggs, egg size, nestling size, and liver biochem istry of nestlings. We chose eastern screech-ow for the study because they were available, read ly breed in captivity (Wiemeyer 1987), and a sensitive to a variety of environmental contam inants (McLane and Hall 1972, Fleming et a 1982, Wiemeyer et al. 1989, Wiemeyer an Sparling 1991).

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imal Care and Use Committee of the Patux- Environmental Science Center.

ETHODS

l Care and Dosing

We randomly assigned to 3 treatments 32 pairs captive eastern screech-owls that hatched in 84 and that produced young in 1987. We gave pairs diets containing no added selenium ontrols) and 4.4 ppm (wet wt) added selenium quivalent to 10 ppm dry wt; low dosage). We ve the remaining 6 pairs a diet containing 2 ppm (wet wt) added selenium (comparable 30 ppm dry wt; high dosage). Five additional irs that hatched in 1984 and that laid in 1987, t produced no young, received the low dosage t. These 5 pairs were used to examine within- tch variation in selenium residues and are reafter referred to as clutch variation pairs.

A dietary dosage of 10 ppm (dry wt) ap- oximated the average concentration (about 11 pm) of selenium found in small mammals at sterson (Clark 1987) and was only slightly her than the mean concentrations in breast ue of ducks and coots at Kesterson (Ohlen- rf et al. 1986). Also, the dietary concentrations re comparable to those given to mallards einz et al. 1987) and black-crowned night- rons (Smith et al. 1988) in experimental stud- s.

We dissolved selenium, in the form of seleno- L-methionine (98% pure; Bachem, Inc., Tor- nce, Calif.), in distilled, deionized water and ixed it in Nebraska Brand Birds of Prey Diet Central Nebraska Packing, North Platte, Nebr.) that 1% water was added to the diet. Controls o received 1% water added to their diet. We ed selenomethionine because it was consid- ed a good model for environmental exposure other studies (Heinz et al. 1989, Hamilton et l. 1990). We blended vitamins, minerals, and antibiotic into the diets (Wiemeyer et al. 989), and formed 25-g meatballs that we froze fore feeding. Each pair received 100 g of food r day. Dietary samples of feed, 1 from each tch mixed (except 2 control batches), con- ined the following concentrations of selenium residue analysis: control ($n = 4$) range 0.092–0.13 ppm (wet wt), <0.21–0.34 ppm y wt); low dosage ($n = 12$) range 3.3–3.9 ppm et wt) $\bar{x} = 3.53$ ppm, range 8.0–9.8 ppm (dry t) $\bar{x} = 8.81$ ppm; high dosage ($n = 3$) all 12 pm (wet wt), 28–31 ppm (dry wt) $\bar{x} = 30$ ppm.

Beginning on 7 January 1988, we housed owl pairs in breeding pens (Wiemeyer 1987). We began feeding treated diets on 13 February. We removed uneaten food and recorded consumption daily, and summarized consumption by 1-week periods. We determined mass of each bird to the nearest g with a Pesola spring scale on 12–13 February and when killed at the end of the study. During the first week, a predator killed 1 adult male owl in the low dosage group thereby reducing the number of pairs on that treatment to 12. We kept the 5 clutch variation pairs that did not produce young in 1987 on treated diet until we killed them. In contrast, we maintained the other pairs on treated diets only until each clutch hatched. Because owls must receive a whole animal diet in order to raise their young (Wiemeyer 1987), we then provided day-old domestic chicks to the pairs 5 days per week and laboratory mice 2 days per week from hatching of the first egg until the youngest nestling in each brood was 5 days old.

Reproductive Effects

We examined each nest box daily from late February through clutch completion. We numbered each egg and collected the second egg laid by each pair 7 days following the initiation of incubation. We examined nest boxes weekly following clutch completion, until 2 days before the projected hatch date when we resumed daily monitoring. We removed unhatched eggs and dead nestlings. We marked each nestling with waterproof ink on the down on top of its head or wings, and weighed them to the nearest g with a Pesola spring scale. We collected all nestlings at 5 days of age, except we took only 1 nestling from 7 randomly selected control broods.

We killed 5-day-old nestlings because mortality related to selenium exposure via the egg was probably complete at this time. Except for 7 control pairs, we then killed the adults and remaining young. We killed pairs when incubation had clearly ceased, when no viable eggs remained, or when all young had died. We killed adults and nestlings by CO_2 asphyxiation, and the last owls died on 20 May 1988. We retained the liver of each adult for selenium analysis. We retained the 5-day-old nestlings for measurement of body components, examination for deformities, and biochemical analyses.

We collected all first clutch eggs from the 5 clutch variation pairs 2 days before the projected hatch date and retained them for deter-

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mination of within-clutch variation of selenium concentrations and examination of embryos for deformities. We killed these pairs at this time and retained the livers for selenium analysis.

We measured length and breadth of the second egg laid by each randomly assigned pair, determined mass, determined volume by water displacement, and retained contents for selenium analysis. We airdried eggshells for a minimum of 30 days after which we determined shell thickness and mass. In eggs that failed to hatch, we determined stage of embryonic development. We examined nestlings and some embryos with a dissecting microscope for external, soft tissue, and skeletal deformities. We cleared nestlings and stained them with alizarin red S (Karnofsky 1965) for skeletal examination and measurement of bone growth.

Liver Biochemistry

We homogenized aliquots of minced liver (1:10 mass/vol) from 5-day-old nestlings in an ice-cold solution containing 1.15% KCl, 0.01M Na K phosphate buffer (pH 7.4), and 0.02M ethylenediamine tetraacetic acid (EDTA). We centrifuged the homogenate at 10,000x *g* for 20 minutes at 4 C and used the supernatant for assays. We recorded liver glutathione peroxidase (GSH peroxidase EC 1.11.1.9; coupled reaction at 30 C with glutathione reductase using cumene hydroperoxide) spectrophotometrically by micromethods using a centrifugal analyzer (Jaskot et al. 1983). We determined crude homogenate and 10,000x *g* supernatant protein concentrations (Lowry et al. 1951) using bovine serum albumin as a standard. We determined reduced glutathione and oxidized glutathione by the method of Tietze (1969) as modified by Griffith (1980) for GSSG using vinyl pyridine. We measured the total hepatic sulfhydryl concentration (total SH; Sedlak and Lindsay 1968), and measured malondialdehyde concentration as an estimate of hepatic lipid peroxidation using the thiobarbituric acid method (Aust 1985). We generated standard curves for the assay using malondialdehyde tetraethyl acetal.

Residue Analysis

We used the method of Krynitsky (1987) in the determination of selenium residues in eggs, tissues, and diet samples. We determined the concentration of zinc in a sample of the control diet (Haseltine et al. 1981), and another sample of control diet was analyzed (Hazleton Laboratories America, Inc., Madison, Wis.) for cystine, methionine, and vitamin E because selenium may be more toxic when concentrations of these dietary constituents are inadequate. The samples contained 45 ppm zinc, 1.83 mg/g cystine, 3.24 mg/g methionine, and 1.63 IU/100g of vitamin E (all wet wt). With the exception of zinc, these concentrations exceed those reported in the supplemented diet of captive mallards (Hoffman et al. 1991*a*) and are considered adequate for eastern screech-owls.

We report selenium concentrations in eggs and liver samples on a wet weight basis. Mean percent moisture values were 60 (n = 19, SE = 0.25) for feed, 80 (n = 22, SE = 0.29) for eggs, and 71 (n = 57, SE = 0.26) for livers. Residue concentrations in eggs and livers cited from published studies are on a wet weight basis unless designated as dry weight. We transformed contaminant concentrations in eggs and livers to logs values to correct for skewness and equalize variance among groups. We report retransformed values (geometric means).

Statistical Analysis

We conducted all statistical comparisons at α = 0.05. We analyzed food consumption data using a general linear model with week included as a repeated measure and pen as a nested factor. We based the test of treatment differences on variation among pens. We excluded pens where 1 bird died during the first few weeks of the study. We compared adult owl body and liver mass data using 2-way analysis of variance (ANOVA) and used a Bonferroni multiple-comparison test to separate means. We used 1-way ANOVA to examine treatment effects for: clutch initiation dates; numbers of eggs laid, incubated, and hatched; nestlings alive at 5 days of age; egg size and shell thickness; and selenium residues in eggs. We subjected percentages of eggs hatched and nestlings alive to arc-sine transformation before using 1-way ANOVA to compare treatments. Differences among treatments were identified with Tukey's multiple comparison procedure. We used *G* tests to compare the proportion of pairs in the treatments successfully completing various aspects of reproduction. We used *t*-tests to compare nestling size and mass, and nestling liver biochemistry parameters for controls and low dosage.

Except for food consumption, we excluded the clutch variation pairs from the above analyses. We used 2-way ANOVA to compare selenium concentrations in livers of adult owls, including data from the clutch variation pairs. We included the following treatment groups in the analysis: control, low dosage (including clutch variation pairs), and high dosage, all on constant exposure at time of sacrifice; and low dosage that had been on clean food ≥5 days at time of sacrifice. We used a Bonferroni multiple-comparison test to separate means.

Before initiation of the study, we examined the issue of statistical power and selected the sample size of control and low dosage pairs that would provide adequate power. At the end of the study we examined the transformed data on percent hatch, an important response. With a power of 0.8, we could have detected a difference between controls and low dosage of 50% or greater.

Table 1. Mass of adult eastern screech-owls fed a control diet or diets containing 4.4 or 13.2 ppm (wet wt) selenium as selenomethionine at the Patuxent Environmental Science Center, 1988.

Selenium added to diet (ppm)	Mass (g)											
	Males						Females					
	Onset of treatment			Sacrifice			Onset of treatment			Sacrifice		
	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE
0	13	182	5	6[a]	154	2	13	219	9	6[a]	200	11
4.4	13	176	5	11[b]	156	3	13	209	6	13	196	4
13.2[c]	6	183	6	5	143	6	6	208	10	5	162	8

[a] Only mass of sacrificed birds determined.
[b] Of the original 13, 1 killed by predator and 1 with missing data.
[c] One of each sex died on the 20th day of treated diet.

RESULTS

Food Consumption

We could not accurately quantify consumption for owls receiving the high dosage because they tended to shred their meatballs which made recovery difficult. Shredding of meatballs was unnoticed during weeks 1 and 2, further complicating interpretation. Pairs on other treatments rarely, if ever, shredded their meatballs. Therefore, we excluded the high dosage from statistical analyses of food consumption. Food consumption during the first 7 weeks was similar (F = 0.32, 1 df, P = 0.576) between controls and those on the low dosage but differed among weeks (F = 2.99, 6 df, P = 0.009). Consumption by pairs on low dosage and the clutch variation pairs on the same treatment was similar during the first 7 weeks (F = 0.59, 1 df, P = 0.453), with a suggestion of a weekly fluctuation in the first 7 weeks (F = 2.10, 6 df, P = 0.061).

Adult Body and Liver Mass

Adult body mass was similar among treatments at the onset of treatment (P = 0.429; Table 1). Female mass was greater (P < 0.0001) than male mass. At sacrifice, differences occurred among treatments (P = 0.0003) and also between sexes (P < 0.0001). Mass of birds that received high dosage ($\bar{x}$ = 152 g, SE = 5.1) was lower than the controls ($\bar{x}$ = 177 g, SE = 8.7) and those on low dosage ($\bar{x}$ = 178 g, SE = 4.9). Change in mass and percent change in mass from the onset of treatment to sacrifice differed among treatments (P = 0.0003 and P < 0.0001), because the high dosage birds lost more mass than the other groups. Change and percent change in mass did not differ between sexes.

Although liver mass was similar among dose groups (P = 0.26), females had larger livers than males (P < 0.0001). We noted similar results when liver mass was expressed as a proportion of body mass.

Selenium in Livers

Geometric mean selenium residues in liver of adults differed among treatments (P < 0.0001) and were different between each treatment (Table 2). Residues were similar (P = 0.779) between sexes, although an interaction (P = 0.0031) between treatment and sex indicated that females had higher concentrations than males only in the high dosage group. Owls that had been off dosage for ≥5 days at time of sacrifice ($\bar{x}$ = 7.3, extremes 5–9 days) had lower (P < 0.05) selenium concentrations in liver than those on constant dosage (Table 2). Two owls that re-

Table 2. Geometric mean selenium concentrations (ppm wet wt) in livers of adult eastern screech-owls fed a control diet or diets containing 4.4 or 13.2 ppm (wet wt) selenium as selenomethionine at the Patuxent Environmental Science Center, 1988.

Selenium added to diet (ppm) and degree of exposure at sacrifice		Males			Females		
		n	$\bar{x}$[a]	SE[a]	n	$\bar{x}$[a]	SE[a]
0	constant	6	0.69A[b]	0.01	6	0.57A	0.03
4.4	off dosage ≥5 days	9	2.2B	0.02	9	2.2B	0.02
4.4	constant[c]	8	5.2C	0.04	9	5.4C	0.03
13.2	constant[d]	4	11. D	0.07	4	17. D	0.08

[a] Standard errors on a $\log_{10}$ basis.
[b] Geometric means for a given sex that are followed by a common letter are not different ($P > 0.05$) from one another.
[c] Includes data from clutch variation pairs that did not produce young in 1987 and so were not randomly assigned to the treatment group (see text), as well as randomly assigned pairs that never went off treated food because their clutches did not hatch.
[d] Pairs were on constant dosage because their clutches did not hatch.

ived the high dosage and were killed at the d of the study after being off treatment for 3 days had 4.4 and 4.7 ppm selenium in their livers.

The 2 owls that received the high dosage and died early in the study had much lower concentrations of selenium in their livers (5.1 and 6.1 ppm) than the mean concentrations in livers of owls from the same treatment on constant dosage that were killed at the end of the study. Both birds were emaciated (male 109 g; female no weight taken, partially eaten by mate), with enlarged kidneys, thickened pericardial sacs (male with fluid in sac), and pale livers with orange coloration. The ventriculus of the male was hemorrhagic.

Egg Size and Selenium Residues

Mean breadth of eggs differed among treatments ($F = 5.54$, 2 df, $P = 0.0099$). Eggs laid by pairs on high dosage were smaller ($P < 0.05$) an that of both controls and those on low dosage (Table 3). Other egg size parameters, shell thickness, and mass did not differ among treatments. Geometric mean selenium concentrations in eggs differed ($F = 244.24$, 2 df, $P < 0.0001$) among treatments, with each treatment different ($P < 0.05$) from each other. Between clutch variability in selenium residues in eggs (63% of the total variation), using data only from the clutch variation pairs where the majority of the clutch was analyzed, exceeded within-clutch variability. No clear trend in selenium residues in eggs was noted in relation to order of laying.

Reproduction

Reproductive success of high dosage pairs was depressed ($P < 0.05$) from that of controls for several parameters (Table 4). Control and low dosage pairs did not differ in reproductive parameters. Only 2 of 13 clutches from control pairs had ≥1 eggs with dead embryos ≥7 days of age. Two of 3 high dosage pairs that incubated their clutches, had ≥1 eggs with dead embryos ≥7 days of age, whereas no development was detected in any egg from the remaining clutch. No clutches of low dosage pairs had eggs with dead embryos ≥7 days of age.

Table 3. Size and selenium residues of eggs produced by eastern screech-owls fed diets containing 0.0 (control), 4.4, or 13.2 ppm (wet wt) selenium as selenomethionine at Patuxent Environmental Science Center, 1988; data restricted to second egg laid in each clutch.

Egg variable	Control (n = 13)		4.4 ppm (n = 12)		13.2 ppm (n = 4)		
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	P[a]
Length (cm)	3.55	0.02	3.50	0.02	3.51	0.05	0.364
Breadth (cm)	3.01A[b]	0.03	3.04A	0.02	2.89B	0.04	0.0099
Egg mass (g)	17.12	0.38	17.40	0.18	16.00	0.28	0.079
Egg volume (mL)	16.69	0.38	16.92	0.23	15.50	0.29	0.093
Shell thickness (mm)	0.226	0.004	0.237	0.007	0.242	0.010	0.261
Shell mass (g)	1.276	0.027	1.351	0.042	1.298	0.039	0.302
Selenium (ppm wet wt)[c]	0.26A		2.57B		7.44C		0.0001

[a] Among treatment effects.
[b] Means for a given variable followed by a common letter are not different ($P > 0.05$) from one another.
[c] Geometric means; n = 6, 12, and 4. Standard errors on a $\log_{10}$ basis are 0.049, 0.027, and 0.076.

Table 4. Reproductive outcome of captive eastern screech-owls given diets containing 0.0 (control), 4.4, and 13.2 ppm (wet wt) selenium as selenomethionine at Patuxent Environmental Science Center, 1988.

Reproductive criteria	Treatment			P[a]
	Control	4.4 ppm	13.2 ppm	
Total pairs	13	12[b]	6	
Pairs laying	13	12	4[c]	0.027
Pairs incubating	13	12	3	0.122
Pairs with hatchlings[d]	13	9	1	0.011
Pairs with 5-day young	12	9	0	0.038
Mean change (days) in laying first egg[e]	+5.8A[f]	+3.6A	+20.8B	0.0004
Eggs laid/pair	4.5A	4.4A	2.8B	0.024
Eggs incubated/laying pair[g]	3.4	3.5	2.5	0.191
Eggs hatched/incubating pair	2.5A	2.5A	0.3B	0.043
Nestlings surviving to 5 days/pair hatching[h]	2.4A	3.3A	0.0B	0.019
5-day-old young/pair	2.4A	2.5A	0.0B	0.0017
% eggs incubated of those available for incubation	100A	100A	85B	0.038
% hatch of eggs incubated	85A	74AB	4B	0.030
% nestlings surviving to 5 days of those hatched	99A	100A	0B	0.0011
% eggs available for incubation producing 5-day young	81A	74A	0B	0.0051

[a] Among-treatment effects.
[b] Male of 1 additional pair killed by predator during the first week of the study.
[c] One member of each of 2 pairs died.
[d] Significant difference between controls and 4.4 ppm selenium ($P = 0.028$).
[e] Laying date of 1988 minus laying date of 1987.
[f] Means followed by letters not in common for a specific parameter are different from one another ($P < 0.05$).
[g] Eggs removed for analysis not included.
[h] Difference between controls and 4.4 ppm selenium approaches significance (t-test; $P = 0.065$).

Nestling Size and Liver Biochemistry

Mass of nestlings on the day of hatch, based on pen means, was similar between controls and low dosage ($t = -0.75$; 20 df; $P = 0.462$); mean mass of control nestlings (n = 13 clutches) was 12.6 g (SE = 0.37), whereas that of low dosage nestlings (n = 9) was 12.9 g (SE = 0.27). Mass of the only high dosage nestling was 12 g on day of hatch. This nestling, which died within 1 day of hatch, was nearly devoid of down, whereas all nestlings from other treatments (33 control and 30 low dosage) appeared normal.

Femur lengths of 5-day-old control nestlings exceeded ($t = 2.6877$, 19 df, $P = 0.0146$) those of low dosage nestlings (Table 5), whereas lengths of other body components and mass of body and liver did not differ between these 2 groups. We noted no malformations of nestlings or embryos in any treatment.

We found a number of liver biochemical abnormalities indicative of oxidative stress ($P < 0.05$) in 5-day-old nestlings from parents fed 4.4 ppm selenium (Table 6). These included a 19% increase in glutathione peroxidase activity, a 21% increase in oxidized glutathione (GSSG), a 43% increase in the ratio of GSSG to reduced glutathione (GSH), a 17% increase in lipid peroxidation (malondialdehyde) and a marginal decrease of 15% in GSH.

Table 5. Mean length (mm) of body components, total body mass (g), and liver mass (g) of 5-day-old nestling eastern screech-owls that hatched from eggs produced by parents receiving diets containing 0.0 (control) or 4.4 ppm (wet wt) selenium as selenomethionine; 1 nestling per brood, first or 1 of several that hatched on the first day of hatching; study conducted at Patuxent Environmental Science Center, 1988.

Variable	Control (n = 12)		4.4 ppm (n = 9)		
	$\bar{x}$	SE	$\bar{x}$	SE	P[a]
Humerus	15.5	0.3	15.1	0.4	0.441
Radius-ulna	14.0	0.2	13.7	0.3	0.354
Femur	20.0	0.4	18.6	0.4	0.015
Tibio-tarsus	20.3	0.4	19.1	0.4	0.058
Crown-rump	95.7	1.4	93.6	1.1	0.266
Body mass	47.3	2.0	46.2	2.7	0.746
Liver mass	2.63	0.14	2.66	0.22	0.901

[a] Between-treatment effects.

Table 6. Effects of selenium on mean liver biochemistry of 5-day-old nestlings of eastern screech-owls that were fed a control diet or one containing 4.4 ppm (wet wt) selenium as selenomethionine at Patuxent Environmental Science Center, 1988.

Variable	Control (n = 12)		4.4 ppm (n = 9)		P[a]
	$\bar{x}$	SE	$\bar{x}$	SE	
GSH peroxidase[b]	182	7	217	8	0.0052
GSH (μmol/g)	3.4	0.2	2.9	0.2	0.059
GSSG (nmol/g)	393	27	474	29	0.046
GSSG:GSH (%)	5.1	0.4	7.3	0.3	0.001
Total SH (μmol/g)	14.4	0.4	14.1	0.4	0.463
Malondialdehyde (nmol/g)	18.6	1.5	21.8	2.4	0.001
Protein (mg/g)	145	1	142	2	0.306

[a] Between-treatment effects; t-tests.
[b] nmol NADPH oxidized/min·mg of 10,000 × g supernatant protein.

DISCUSSION

Food Consumption and Avoidance

The deaths of the 2 owls that received high dosage may have been due to food avoidance. Shredding of meatballs by owls receiving this diet was abnormal and was not seen in other groups. This behavior suggests that they were able to detect the selenium in the diet. Heinz and Sanderson (1990) considered food avoidance by adult mallards given dry diets containing 10 and 20 ppm selenium as selenomethionine to be a conditioned response, probably related to post-ingestional illness, rather than a reaction to the taste of the chemical.

Due at least in part to avoidance of the treated food, owls on the high selenium diet lost considerable body mass which may have adversely affected their health and contributed to subpar reproduction. Loss of body mass also occurred in experimental studies of mallards (Heinz et al. 1989) and black-crowned night-herons (Smith et al. 1988; Table 7).

Variation in food consumption among weeks was possibly due to ambient weather conditions in the outdoor pens. Similar food consumption between low dosage pairs and the clutch variation pairs on the same treatment indicates that selenium exposure between the 2 groups was similar.

Selenium in Livers

The equation presented by Heinz et al. (1989) predicting selenium residues in livers of female mallards from dietary concentrations provided a reasonable estimate for female owls in our study, whereas the estimate for male mallards greatly exceeded that found in the livers of male owls. Livers of female mallards fed a diet containing 10 ppm selenium as selenomethionine had similar concentrations of selenium as both sexes of owls receiving a similar dietary concentration (Table 7), whereas livers of male mallards contained much higher concentrations (Heinz et al. 1987). The difference between male and female mallards may have been due to females excreting selenium through laying large numbers of eggs. Livers of black-crowned night-herons (*Nycticorax nycticorax*) that received diets containing similar concentrations of selenomethionine as our owls had similar concentrations of selenium as livers of our owls (Table 7).

Selenium concentrations in the livers of 2 high-dosage owls that died were far below those associated with selenium toxicosis in other species (Ohlendorf et al. 1988*a*), suggesting that the cause of death may have been related to starvation and not selenium toxicosis.

Limited data are available on selenium residues (dry wt) in livers of raptors from the Kesterson area. Two juvenile northern harriers (*Circus cyaneus*) contained 22 and 24 ppm (Ohlendorf et al. 1990). The liver of a nestling red-tailed hawk (*Buteo jamaicensis*) contained 5.3 ppm (U.S. Bur. Reclamation 1990) and the livers of 2 adult barn owls (*Tyto alba*) contained 3.6 and 12.1 ppm (U.S. Bur. Reclamation 1989). The harrier livers contained slightly higher concentrations of selenium than owls from the low dosage group that were on constant dosage (about 18 ppm dry wt), whereas the remaining wild birds had much lower concentrations of selenium.

Owls rapidly eliminated selenium from their livers as shown by the lower selenium levels in owls from the low dosage group that were off dosage for ≥5 days at sacrifice as compared to those of owls on constant dosage (Table 2). Slightly greater than ½ of the selenium was lost during this period. Rapid elimination of selenium from livers also occurred in mallards (Heinz et al. 1990). Female mallards maximally dosed with selenium in the form of selenomethionine lost ½ of the selenium in their livers in 3.3 days following cessation of dosage, whereas additional 50% decreases occurred in 3.9 and 6.0 days. Female mallards given a diet containing 10 ppm selenium in the form of selenomethionine lost ½ of the selenium in their livers in 18.7 days following cessation of dosage.

Table 7. Sensitivity of 3 species of captive birds to the effects of dietary selenomethionine.

Variable	Se exposure (ppm dry wt)	Species: Mallard[a]	Black-crowned night-heron[b]	Eastern screech-owl[c]
Adult weights	10	No effect	No effect	No effect
	30	NT[d]	Significant loss	Significant loss
Se in adult livers (ppm wet wt)				
Male	10	8.6	5.1	5.2
	30	NT	17.	11.
Female	10	4.7	4.2	5.4
	30	NT	13.	17.
Teratogenic effects	10	Significant incidence	No effect	No effect
Hatching success	10	No effect	No effect	No effect
Chick liver biochemistry[e]	10	NT	Malondialdehyde increased	Malondialdehyde increased
Se in eggs (ppm wet wt)	10	4.6	3.3	2.6
	30	NT	9.2	7.4

[a] From Heinz et al. (1987) and Hoffman and Heinz (1988).
[b] From Smith et al. (1988).
[c] From this study.
[d] NT = not tested.
[e] Indicators of lipid peroxidation.

Reproductive Effects

Screech-owls were less sensitive to selenomethionine than mallards and similar in sensitivity to black-crowned night-herons as measured by teratogenic effects and hatching success (Table 7). Additional effects of importance were also noted. Mallards fed a dry diet containing 10 ppm selenium as selenomethionine also produced fewer ducklings per female than controls (Heinz et al. 1987). Mallards that were fed dry diets containing 8 and 16 ppm selenium as selenomethionine produced 4.6 and 0 young to 6 days of age per female, whereas controls produced 8.1 young per female (Heinz et al. 1989). The prevalence of malformations in embryos of eggs from mallards fed diets containing 8 and 16 ppm selenium as selenomethionine were about 10 and 100 times that of controls. Owls fed high dosage (30 ppm, dry wt, selenium) had markedly reduced reproductive success with smaller eggs than those of controls. Five-day-old screech-owl nestlings from parents that received low dosage had shorter bone lengths than controls. These effects were also reported in 3-day-old nestlings of black-crowned night-herons whose parents had received a similar dietary concentration of selenomethionine (Smith et al. 1988).

Hepatic oxidative stress caused by excess dietary selenium, including higher GSH peroxidase activity, GSSG concentration, ratio of GSSG to GSH, depleted hepatic thiol concentration, and lipid peroxidation that was seen in screech-owl nestlings has also been reported in mallard ducklings (Hoffman et al. 1989, 1991*b*, 1992). These effects became more pronounced in mallard ducklings with increasing dietary concentrations of selenomethionine, eventually resulting in decreased growth, histopathological lesions, and mortality and, therefore, serve as good indicators for the onset of selenium-related toxicosis.

Screech-owls fed diets containing concentrations of selenomethionine similar to that of other species laid eggs with lower concentrations of selenium (Table 7). Also, mallards fed dry diets containing 8 and 16 ppm laid eggs containing means of 11 and 18 ppm selenium (Heinz et al. 1989). The higher selenium concentrations in eggs of mallards may explain, in part, their greater sensitivity in relation to dietary concentrations. However, differences in sensitivity among species may also occur.

The mean concentration of selenium in eggs

of owls on low dosage (12.9 ppm dry wt converted from wet wt) was higher than concentrations (dry wt) in eggs of wild raptors from selenium contaminated areas of the western United States. Six northern harrier eggs from Kesterson contained 1.1 to 9.8 ppm selenium (U.S. Bureau of Reclamation 1990, 1993; Ohlendorf and Hothem 1995). A short-eared owl (*Asio flammeus*) egg from Sun River, Montana had 3.4 ppm and 2 northern harrier eggs from Benton Lake, Montana had 2.4 and 3.9 ppm selenium (Lambing et al. 1994). A northern harrier egg from Middle Green River, Utah had 5.2 ppm selenium (Peltz and Waddell 1991).

MANAGEMENT IMPLICATIONS

Our results demonstrate that potential threats of selenium toxicity to reproduction of raptors should be considered where raptors frequent areas of selenium contamination. In the degree of cleanup of such areas managers should consider not only selenium concentrations in aquatic, but also terrestrial ecosystems. Areas where breeding raptors are feeding on prey contaminated with high levels of selenium should receive close scrutiny to insure that selenium concentrations in these prey do not exceed 10 ppm dry weight (4.4 ppm wet wt). At this dietary concentration in captive adult eastern screech-owls, hepatic oxidative stress was noted in nestlings, which is an indicator for the onset of selenium-related toxicosis.

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