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SELENIUM ACCUMULATION IN CAPTIVE AMERICAN KESTRELS (*FALCO SPARVERIUS*) FED SELENOMETHIONINE AND NATURALLY INCORPORATED SELENIUM

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Abstract—Male–female pairs of kestrels were maintained for 11 weeks on diets containing 5 or 9 ppm selenium (Se) (dry weight) as seleno-L-methionine, or naturally incorporated Se in the form of mammals collected at Kesterson Reservoir, CA, USA. Selenium concentrations in blood and excreta of male and female kestrels within groups were similar. Near-maximal mean Se concentrations in blood were observed after the 5th week of treatment in the seleno-L-methionine-treated kestrels, and an approximately 1:1 ratio was observed between maximal blood concentrations and dietary concentrations. All treatment groups exhibited reduction of Se concentration in excreta, but not in blood, to baseline values 4 weeks after treatment ended. No birds were observed to exhibit signs of general illness or Se toxicity during the study.

Keywords—Selenium Selenomethionine Bioaccumulation Depuration Kestrel

INTRODUCTION

During the early to mid-1980s, significant losses of aquatic birds at Kesterson Reservoir (Merced County, CA, USA) were attributed to elevated selenium (Se) levels originating from agricultural drainage [1]. Similar situations exist in other areas of California and in other western states, where irrigation practices lead to high concentrations of Se and other trace elements in drainwater [2]. Since 1987, monitoring of terrestrial mammals and birds at Kesterson Reservoir (Kesterson) has not indicated reductions in reproduction due to Se, in spite of persistently elevated Se levels in soil, plants, invertebrates, and vertebrates, relative to reference sites [3]. As part of the mitigative effort carried out at Kesterson, the reservoir was drained, low elevation areas were filled, and it was transformed into an early successional terrestrial habitat in 1988.

Reproductive effects of Se reported in wild aquatic birds include embryo mortality and teratogenesis [1,4]. Such effects have been produced in laboratory dietary studies using both organic and inorganic forms of Se (mallards, *Anas platyrhynchos* [5,6], and domestic chickens [7,8]). Selenomethionine, the most bioavailable and toxic of the different Se compounds studied, and selenite are associated with reproductive effects in mallards at dietary concentrations as low as 7 and 2 ppm dry weight, respectively [5,6,9,10]. The occurrence of similar concentrations of Se in plant, invertebrate, and vertebrate dietary items currently found at Kesterson suggests a possibility for reproductive impairment in breeding birds that forage extensively in this area [3].

Lack of detection of impaired breeding or other adverse effects in terrestrial avian species may be due in part to the limited area of Kesterson (517 hectares) and relatively small numbers of nesting terrestrial birds available for analysis. Alternatively, sensitivity and/or exposure patterns of terrestrial species currently monitored at this site may differ from that of previously studied aquatic birds. Although a relatively large database exists on the effects of Se on waterfowl, shorebirds, and domestic bird species, such information on wild terrestrial birds is limited [1]. To investigate whether dietary Se concentrations found at Kesterson can cause reproductive effects in terrestrial avian species, laboratory feeding studies were undertaken using the American kestrel (*Falco sparverius*), an avian predator that forages at Kesterson. This paper describes pilot data on the accumulation and depuration of Se in blood and excreta of breeding kestrels fed either seleno-L-methionine in the diet or naturally occurring selenium in the form of small mammals collected at Kesterson.

METHODS

Animals and treatments

American kestrels were purchased from a captive colony at McGill University (Montreal, Quebec, Canada) and housed at the University of California at Davis (UCD) in large indoor flight pens. Kestrels were acclimated to these conditions for at least 1 month prior to the onset of the study. Kestrels were cared for according to animal care protocols approved by the Office of the Campus Veterinarian at UCD.

After acclimation, male and female kestrels were paired and randomly assigned to treatment groups. Individual pairs were housed in approximately $2 \times 2 \times 1.75$ m pens, which were maintained at ambient temperature and photoperiod in a large, covered building with screen siding. Each pair was provided with about 100 g of fresh food daily, and tap water was provided ad libidum. Each pen was equipped with a wooden nest-box and perches.

Dietary treatments were initiated 1 week after introduction to the pens. Control birds ($n = 9$ pairs) received a diet of killed cockerels (Ward and Drosky Hatchery, Santa Cruz, CA, USA), laboratory mice (UCD), and commercial raptor chow

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(Nebraska® Bird of Prey Diet, Central Nebraska Packing, North Platte, NE, USA). A second group (site-fed group, n = 10 pairs) received their entire diet as mammals collected at Kesterson. For this group, small- to medium-sized mammals, including California voles (*Microtus californicus*), deer mice (*Peromyscus maniculatus*), California ground squirrels (*Spermophilus beecheyi*), desert cottontails (*Sylvilagus audubonii*), and black-tailed hares (*Lepus californicus*), were trapped or shot at Kesterson, frozen in plastic bags, and transported to UCD, where they were frozen until just prior to feeding. Pairs dosed with seleno-L-methionine were fed commercial raptor chow with seleno-L-methionine (Sigma, St. Louis, MO, USA) mixed in at concentrations of 5 (low Se, n = 10 pairs) or 9 (high Se, n = 10 pairs) ppm Se (dry weight). Doses were selected to mimic dietary levels experienced by raptors foraging 50% (low Se) or near-100% (high Se) of the time on small mammals and invertebrates at Kesterson. For mixing, seleno-L-methionine dissolved in triple-distilled water and a vitamin supplement (Vionate®) were blended into the diet using a Hobart® mixer. Moisture added to the diet by the seleno-L-methionine solution was less than 1%. The control diet was prepared in a similar fashion, using triple distilled water instead of seleno-L-methionine solution. Birds were exposed to treated diets for 77 d during the breeding season (June 7–September 6). After that time, they were changed over to control diet.

Sample collection and selenium analysis

Blood samples (approximately 0.2 ml) were drawn from birds prior to treatment and at 14, 35, 56, 77, 91, and 105 d after the onset of treatments. Blood was drawn from the brachial vein into heparanized insulin syringes and frozen in plastic microcentrifuge tubes. At the time of blood sampling, birds were grossly examined and weighed to assess general condition. Female kestrels exhibited highly variable body weights over the study period, depending on their reproductive condition. Body weight increases of up to 48% (unpublished data) over the reproductive period in individual females, which did not breed synchronously within the colony, made it impossible to isolate body weight changes not related to reproduction during this time. Therefore, only body weights of male kestrels were considered in treatment comparisons. Fresh excreta samples (approximately 1 g) were collected at blood collection times and also frozen in microcentrifuge tubes. Because male and female excreta from the same pens were not distinguishable, samples from each pen were considered as pooled samples for male and female pair members. Diet samples were collected periodically during the study. Portions of commercial diet were collected each time a control, low-Se or high-Se treatment diet batch was mixed, as were livers from all mammals used for the site-fed group. Three laboratory mice and one cockerel were also collected. Blood, excreta, and diet samples were stored at −70°C, then shipped to a commercial laboratory (Laboratory of Environmental Testing, Columbia, MO, USA) on dry ice for total Se analysis. Samples were lyophilized and then analyzed for Se content by graphite furnace atomic absorption spectrophotometry as described by the U.S. Bureau of Reclamation [11]. Lyophilization data were used to calculate percent moisture in all samples (average moisture was 80%). Whole carcass Se concentrations were estimated for mammals based on measured liver Se using a regression formula previously established for mammals collected at Kesterson [3]. Diet samples from Kesterson were analyzed only for Se because prior studies have established that Se is the only contaminant of concern in food chain biota at Kesterson and that other compounds, such as other trace elements and organochlorines, generally occur at levels similar to background (see Ohlendorf and Santolo [3] for review). All Se concentrations are expressed as parts per million on a dry weight basis.

Table 1. Selenium (Se) concentrations in samples of control diet and diets containing seleno-L-methionine (low-Se and high-Se) and naturally incorporated Se (site fed) fed to kestrels for 11 weeks

		Se (ppm, dry wt)	
Dietary item	n	Mean	SD
Control diet[a]	76	1.3	0.24
Low-Se diet	9	5.1	0.64
High-Se diet	9	9.0	0.56
Site-fed diet[b]	517	8.0	5.6

[a] The control diet fed over the 77-d treatment period was made up of 18% mouse (0 = 0.69 ppm Se, n = 3, SD = 0.16), 50% cockerel (2 ppm Se), and 32% raptor chow (0 = 0.59 ppm Se, n = 6, SD = 0.13).
[b] Whole carcass concentrations estimated from liver concentrations.

Statistical analyses

Blood Se data were not normally distributed within treatment groups (Shapiro-Wilk W test [12]). Male–female blood Se concentrations were compared using the Mann–Whitney U test [13]. Comparisons among groups were made using log-transformed data and conducted with one-way analysis of variance and the Tukey–Kramer HSD for multiple means comparisons ($\propto$ = 0.05) [12]. Log-transformed data are reported as geometric means. The relationship between dietary Se concentration and blood Se concentration was analyzed using simple linear regression [13].

RESULTS

Dietary selenium levels

Total Se concentrations were significantly different among treatment diets (Table 1), with the exception of site-fed and high-Se diets, which were similar in Se levels. Within treatments, dietary Se concentrations changed significantly over the course of the study period for only the site-fed group.

Selenium bioaccumulation

For most sample days, Se concentrations in blood of males and females within groups were not significantly different, with exceptions on day 91 in the low-Se (males higher than females) and day 14 in the high-Se (females higher than males) groups (Fig. 1; data shown for control, low-Se and high-Se groups). Subsequently, male and female data within groups were pooled for statistical analyses.

Near-maximal mean Se concentrations in blood were observed by day 35 in the low-Se and high-Se groups (Table 2). A significant positive correlation ($p < 0.0001$) was observed between maximal blood Se concentrations of seleno-L-methionine–treated kestrels and dietary Se concentrations (Fig. 2). Site-fed birds exhibited variable Se concentrations in blood, with a maximal mean concentration on day 56, which was higher than on all other days for this group.

After day 1, low-Se, site-fed, and high-Se groups exhibited higher blood (days 14–105) and excreta (days 14–77) Se concentrations than those of the control group. During the treat-

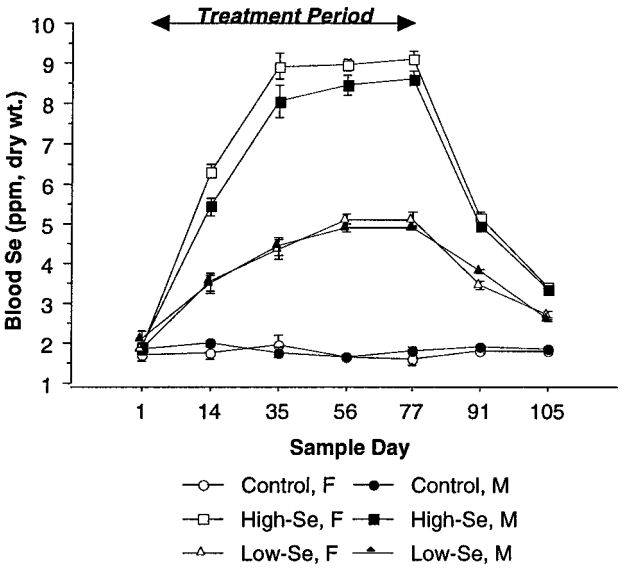

Fig. 1. Blood selenium (Se) concentrations over time in male and female kestrels during and after exposure to dietary seleno-L-methionine (5 or 9 ppm Se) and control diet. Values are shown as the mean ± SE.

ment period (days 14–77), blood and excreta Se concentrations in the high-Se group were significantly higher than those in the low-Se group. Selenium concentrations in blood of the high-Se group were higher than those of the site-fed group on days 35 through 77. There were few differences in excreta Se concentrations between site-fed and high-Se groups during the study, with the exception of day 56, when the excreta Se concentration in the site-fed group was significantly higher than those for all other groups. For both posttreatment samples (days 91 and 105), Se concentration in blood, but not excreta, in all treatment groups remained higher than control concentrations, and blood Se concentrations in the high-Se group remained higher than those of the site-fed and low-Se groups.

Table 2. Geometric mean concentrations of selenium (Se, ppm, dry weight) in blood and excreta of kestrels fed dietary seleno-L-methionine (5 and 9 ppm Se), naturally incorportaed Se (mammals), or control diet for 11 weeks[a]

		Treatment			
Day	Sample	Control[b]	Low-Se[c]	Site-fed[d]	High-Se[d]
1	Blood	1.7A	1.9A	1.7A	1.9A
	Excreta	3.0A	2.8A	2.8A	2.8A
14	Blood	1.9A	3.4B	5.1C	5.8C
	Excreta	2.2BC	5.2B	9.1C	9.4C
35	Blood	1.8A	4.3B	4.6B	8.4C
	Excreta	1.8A	6.6B	10C	12D
56	Blood	1.6A	4.9B	7.5C	8.7D
	Excreta	1.4A	5.8B	19C	12D
77	Blood	1.7A	5.0B	5.9C	8.9D
	Excreta	1.6A	5.2B	9.3C	9.9C
91	Blood	1.9A	3.6B	3.8B	5.8C
	Excreta	2.6A	3.2BC	2.9B	3.7C
105	Blood	1.8A	2.6B	2.6B	3.4C
	Excreta	2.8A	2.9A	2.8A	3.1A

[a] Means sharing the same lower case letters within rows are not significantly different ($\propto = 0.05$).
[b] n = 15 to 18 birds (blood), 8 to 9 pairs (excreta).
[c] n = 17 to 20 (blood), 8 to 9 pairs (excreta).
[d] n = 20 birds (blood), 10 pairs (excreta).

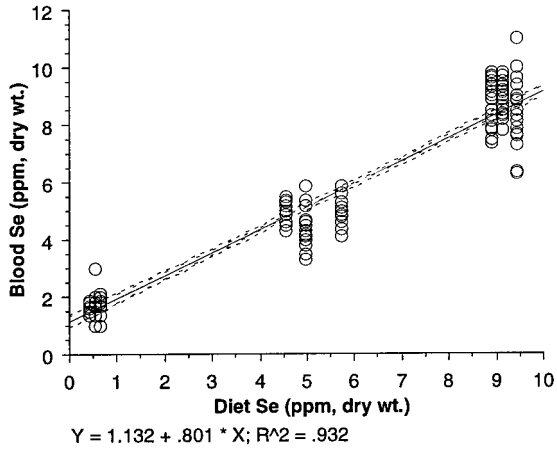

Fig. 2. Linear regression of dietary and maximal blood selenium (Se) concentrations in kestrels exposed to dietary seleno-L-methionine (5 or 9 ppm Se) and control diet. Blood Se concentration values for sample days 35, 56, and 77 were included in the analysis.

Effects in treated kestrels

No birds were observed, at a gross level, to exhibit signs of general illness or Se toxicity during the study. Within groups, there were no significant changes in body weights of male kestrels over time compared with baseline weights, with one exception in the high-Se group. In this group, mean body weight on day 77 was significantly lower than on day 105. One control female and one female and one male from the low-Se group died during the study. Both female birds died due to egg peritonitis, caused by ovum or egg breakage into the body cavity. This condition may have resulted from several events, including oviduct spasm, entry of two ova simultaneously into the oviduct, or physical trauma, such as that caused by handling during sampling ([14], N. Stedman, personal communication). The proximate cause of death in the male kestrel was apparent handling stress (death occurred suddenly within approximately 1 h after blood sampling). Necropsy and Se concentration in liver (2.7 ppm, dry weight) of this bird did not indicate gross congenital abnormalities, physical injuries, or Se toxicosis (e.g., anorexia, liver necrosis, muscle loss, absence of subcutaneous fat [15]).

DISCUSSION

Selenium-containing diets fed to captive American kestrels simulated dietary Se exposure of wild terrestrial birds that currently utilize Kesterson. (In 1994 and 1995, mean Se concentrations in small mammals trapped at Kesterson were about 10 and 6.8 ppm, dry weight, respectively [16].) In addition, blood Se concentrations of treated kestrels bracketed the highest and lowest mean blood Se concentrations found among raptor species sampled at Kesterson [11,16]. Seleno-L-methionine and Se-contaminated small mammals produced similar blood concentrations of Se in kestrels when fed at comparable concentrations of total Se, suggesting similar bioavailability of the two different Se sources in kestrels. Together, field and laboratory data suggest that seleno-L-methionine may be an appropriate form of Se for future studies of Se effects in raptors.

Blood has rarely been used in Se investigations as a tissue for monitoring exposure in bird species [9]. However, this nondestructive method of tissue sampling is desirable in that it allows repeated sampling of individuals and reduces sacri-

ficing of study animals. Particularly in field studies, terminal sampling often is not permissible or practical in many species of interest. In this study, an approximate 1:1 relationship between dietary and peak blood Se concentrations (dry weight basis) was observed in birds receiving seleno-L-methionine in the diet, and the time to reach a plateau of Se in the blood was about 5 weeks. Under a similar exposure regime in mallards, peak concentrations were achieved after a much longer period, 12 weeks, and a greater than 1:1 ratio between blood and diet Se was reported [17]. Based on studies such as these, blood concentration, under conditions of chronic exposure, can facilitate estimation of amount of Se in the diet as well as serve as an indicator of recent exposure.

Selenium concentrations in both raptor prey items and blood at Kesterson are sufficiently high to suggest possible adverse reproductive and other health effects [3] based on studies of captive and wild birds [4,6,18,19]. In eastern screech-owls (*Otus asio*), whereas 10 ppm of Se (as selenomethionine) in the diet did not produce overt toxicity or weight loss in adults, nestlings of treated parents exhibited signs of hepatic oxidative stress and reduced femur lengths compared with controls [20]. Reproductive effects and hepatic oxidative stress have likewise been reported for mallards fed 8 ppm of Se as selenomethionine [6,21]. Interestingly, adverse effects of Se have not been observed in raptors or other terrestrial avian species during ongoing monitoring efforts at Kesterson. Therefore, the risk to wild birds posed by environmental Se contamination at Kesterson remains unknown. Captive kestrels exposed to 5 and 9 ppm dietary Se remained in apparent good health throughout this study, as indicated by subjective evaluation and body weight. Several birds from control and low-dose groups died during the study; however, causes of death in these birds did not appear to be treatment related. Further investigations in kestrels are under way to determine whether reproductive effects occur in this species as a result of exposure to environmentally relevant Se concentrations.

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