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Liver Metal Concentrations in Greater Sage-grouse (*Centrocercus urophasianus*)

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ABSTRACT: Greater Sage-grouse (*Centrocercus urophasianus*) are a species of concern due to shrinking populations associated with habitat fragmentation and loss. Baseline health parameters for this species are limited or lacking, especially with regard to tissue metal concentrations. To obtain a range of tissue metal concentrations, livers were collected from 71 Greater Sage-grouse from Wyoming and Montana. Mean±SE metal concentrations (mg/kg wet weight) in liver were determined for vanadium (V) (0.12±0.01), chromium (Cr) (0.50±0.02), manganese (Mn) (2.68±0.11), iron (Fe) (1,019±103), nickel (Ni) (0.40±0.04), cobalt (Co) (0.08±0.02), copper (Cu) (6.43±0.40), mercury (Hg) (0.30±0.09), selenium (Se) (1.45±0.64), zinc (Zn) (59.2±4.70), molybdenum (Mo) (0.93 ± 0.07), cadmium (Cd) (1.44 ± 0.14), barium (Ba) (0.20 ± 0.03), and lead (Pb) (0.17 ± 0.03). In addition to providing baseline data, metal concentrations were compared between sex, age (juvenile/adult), and West Nile virus (WNv) groups (positive/negative). Adult birds had higher concentrations of Ni and Cd compared to juveniles. In addition, Zn and Cu concentrations were significantly elevated in WNv-positive birds.

Key words: *Centrocercus urophasianus*, ICP-MS, liver, metals, Sage-grouse.

Species-specific normal concentration ranges of metals are valuable diagnostic tools and can be used to identify a variety of disorders, assess the overall health of an animal, and aid in detecting toxicologic hazards within the environment. Trace metal baseline data are sorely lacking for many wildlife species, including Greater Sage-grouse (*Centrocercus urophasianus*). The common practice of using normal values of domestic species for comparison may be misleading due to physiologic or environmental variations (Zaugg and Kinsel, 1997; Blakely et al., 2000). Metal toxicosis is a major problem for many avian species (e.g., lead poisoning in waterfowl due to ingestion of lead shot), and deficiencies of trace elements contribute to increased clinical disease (Osofsky et al., 2001). This survey was conducted to help establish normal ranges of metal concentrations in the liver of Greater Sage-grouse and to determine differences related to sex, age, and the presence of West Nile virus (WNv), an emerging disease of this species in western North America (Clark et al., 2006; Walker et al., 2004).

Greater Sage-grouse ($n=71$) submitted for diagnostic testing to the Wyoming State Veterinary Laboratory (WSVL) during 2003 through 2006 were included in this survey. Fifty-three birds were submitted from seven Wyoming counties including Campbell (19), Fremont (7), Natrona (3), Sweetwater (3), Park (1), Johnson (17), Albany (2), and Big Horn (1). The remaining 18 birds were from five Montana counties including Musselshell (6), Big Horn (4), Fergus (2), Missoula (1), and Phillips (5). All birds received a complete diagnostic necropsy and associated testing, including real-time reverse transcription-polymerase chain reaction (RT-PCR) testing (Shi et al., 2001) and immunohistochemical analysis of brain, heart, and kidney (at a minimum) for WNv (Kiupel et al., 2003). Liver samples for metals analysis were collected during necropsy and frozen (−20 C) until analysis. Nineteen males and 52 females comprised the group, and a total of 57 were adults. Twenty-eight birds were confirmed to be infected with WNv. Trauma was determined to be the cause

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of death for remaining birds (e.g., hit by a car or predation).

A WSVL standard nutritional and toxic metals screen was utilized which included vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), cobalt (Co), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd), barium (Ba), mercury (Hg), thallium (Tl), and lead (Pb). Samples for metals analysis were digested in groups of 12 (nine samples, two quality control samples, and one reagent blank). Approximately 1.0 g of liver was weighed into a Teflon microwave bomb and 3 ml each of concentrated HNO_3 and 30% (v/v) H_2O_2 were added. After setting for approximately 5 min, each bomb was sealed and progressively heated to 180 C in a microwave oven (MDS 2000, CEM Corp, Matthews, North Carolina, USA). Each digestor run included one reference material (NIST 1577b, NIST, Gathersburg, Maryland, USA) and a duplicate liver sample spiked with approximately 50% of the anticipated analyte concentration (laboratory fortified matrix spike). After 10 min at 180 C, samples were cooled and quantitatively transferred to 15 ml polypropylene tubes (#2086; Elkay Plastics Co., Commerce, California, USA) and q.s. to 10 ml with 18 Mohm deionized water. A reagent blank was created by microwaving a 1:1 mixture of HNO_3 and H_2O_2 according to the same protocol. All reagents used were trace element grade and prepared with deionized water (18 Mohm) prepared with a Millique (Millipore, Bedford, Massachusetts, USA) system.

All samples were diluted as follows: One ml of liver digest was combined with 1 ml of internal standard (200 µl/l germanium [Ge], scandium [Sc], yttrium [Y], indium [In], terbium [Tb], and bismuth [Bi]) prepared from 10 mg/l stock solution (SPEX Certiprep, Metuchen, New Jersey, USA) and 4 ml of deionized water in a 15-ml tube. A matrix blank and a set of matrix-matched analytical standards were used in building an external calibration curve. Liver samples were analyzed using an Elan 6100 ICP-MS (PE Sciex, Norwalk, Connecticut, USA).

Statistical software SPSS 13.0 was used for all data analysis (SPSS Inc., Chicago, Illinois, USA). Any values greater than 5 standard deviations from the mean were considered outliers and consequently removed from the data set. Normality was tested using the Kolmogorov-Smirnov test ($P<0.05$) prior to additional statistical analysis. Independent t-tests ($P<0.05$) were used to compare individual metals between WNv groups (positive/negative), sex, and age (juvenile/adult). Collection locations were not compared due to the small sample size per area and the lack of consistent location data (i.e., latitude and longitude). Results for all metals and method detections limits (MDL) are reported on a wet weight basis.

Method detection limits and summary statistics for all metals are shown in Table 1. No liver samples contained Tl and As concentrations above the MDL. A relatively small number of samples contained V, Hg, and Co above their respective MDL, and no significant differences between age, sex, or WNv groups were detected for these elements. There were no significant differences due to age, sex, or WNv status when Cr, Mn, Fe, Se, Mo, Ba, or Pb concentrations were compared ($P>0.05$).

Adult birds had significantly ($P=0.005$) higher Cd concentrations (1.63±0.15 mg/kg) than juveniles (0.70±0.23 mg/kg); however, concentrations were not significantly different between sexes ($P=0.281$) or WNv groups ($P=0.442$). Adult sage-grouse also had significantly ($P=0.001$) higher concentrations of Ni (0.44±0.05 mg/kg) than juveniles (0.23 ± 0.02 mg/kg). Nickel did not differ significantly with sex ($P=0.903$) or WNv status ($P=0.775$).

The higher concentration of Cd in adult sage-grouse is most likely a result of accumulation with age. Cadmium accumulation in the kidney is a primary concern as the metal can cause permanent renal tubular damage (National Research

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TABLE 1. Summary statistics for selected metals detected in liver samples from Greater Sage-grouse collected from Wyoming and Montana, 2003-2006.

Metal	N	MDL[a]	Mean	SE[b]	95% Confidence interval	Min	Max
V	10	0.10	0.12	0.01	0.11–0.14	0.10	0.16
Cr	70	0.05	0.50	0.02	0.47–0.54	0.17	0.80
Mn	70	0.05	2.68	0.11	2.46–2.91	0.86	5.47
Fe	71	0.05	1,019	103	814–1,225	65.5	4,200
Ni	60	0.05	0.40	0.04	0.32–0.47	0.08	1.61
Co	13	0.05	0.08	0.02	0.03–0.13	0.05	0.35
Cu[c]	42	0.05	5.48	0.47	4.53–6.44	1.59	19.4
Zn[c]	43	0.05	44.07	4.13	34.65–50.87	17.1	163
As	71	0.50	BMDL[d]	NA[e]	NA	NA	NA
Se	54	1.00	1.45	0.64	1.32–1.58	1.00	3.20
Mo	71	0.05	0.93	0.07	0.79–1.07	0.10	3.42
Cd	70	0.05	1.44	0.14	1.17–1.71	0.06	5.60
Ba	47	0.05	0.20	0.03	0.14–0.25	0.05	1.03
Hg	10	0.05	0.30	0.09	0.09–0.50	0.07	0.96
Tl	71	0.05	BMDL	NA	NA	NA	NA
Pb	33	0.05	0.17	0.03	0.11–0.23	0.05	0.66

[a] MDL = method detection limit.

[b] SE = standard error.

[c] Only includes WNv-negative grouse.

[d] BMDL = below method detection limit.

[e] NA = not applicable.

Council [NRC], 2005). Larison et al. (2000) found that Cd accumulated at a rate of 0.5 μg per day in white-tailed ptarmigan (*Lagopus leucura*) in a contaminated area in Colorado. Liver samples from white-tailed ptarmigan in control areas contained less than 5 mg/kg Cd (Larison et al., 2000). Liver Cd concentrations in the current study were also less than 5 mg/kg.

Similar to Cd, Ni was higher in adult Greater Sage-grouse as compared to juveniles. However, this difference is not as easily explained because Ni does not accumulate in any organ over time (NRC, 2005). The observed difference may be due to collection location reflecting variation in environmental Ni concentrations. Avian species tend to accumulate Ni from contaminated sites (i.e., areas near smelters; Outridge and Scheuhammer, 1993). Rose and Parker (1983) reported 0.8 mg/kg dry weight Ni in the liver of Ruffed Grouse (*Bonasa umbellus*) from an uncontaminated site. Livers collected from Ruffed Grouse near smelters contained 2.3 mg/kg Ni dry weight. A review by Outridge and Scheuhammer (1993) reported organ concentrations of Ni in most avian species from uncontaminated sites range from 0.1–5 mg/kg dry weight. Using four as the conversion factor from dry weight to wet weight, concentrations of Ni in the current study are well below 3.2 mg/kg of liver found in Ruffed Grouse liver.

Zinc concentration was higher ($P=0.001$) in WNv-positive (82.4±8.54 mg/kg) as opposed to WNv-negative (44.1±4.13 mg/kg) Greater Sage-grouse. Copper concentration was also higher ($P=0.003$) in WNv-positive (7.85±0.61 mg/kg) than in negative (5.48±0.47) birds. No significant differences were observed for sex (Zn $P=0.438$; Cu $P=0.411$) or age (Zn $P=0.293$; Cu $P=0.543$) groups for these two metals.

The significantly higher concentration of Zn and Cu in WNv-positive birds could be a result of the infection. Trace metal metabolism changes in response to disease because certain metals (i.e., Zn and Cu) are cofactors for immune system proteins

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(Wellinghausen et al., 1997; Butcher and Miles, 2002). During an immune response, Zn concentrations decrease in serum and increase in the liver. This sequestered Zn is stored on hepatic metallothionein for production of acute phase proteins (Butcher and Miles, 2002). Zinc also influences the immune function indirectly; for example, the hormone thymulin is thought to regulate natural killer cells, but it is only biologically active when bound to Zn (Oliver and Marsh, 2003). While Zn serum concentration decreases during an immune response, Cu serum concentration increases. Copper is also a cofactor for many proteins involved in the immune system and is essential for an effective immune response (Hogstad, 1996; Arredondo and Núñez, 2005). For example, Cu is a key component in the production of interleukin-2 (Arredondo and Núñez, 2005). Ceruloplasmin, a Cu dependent antioxidant, is also increased in inflammation (Arredondo and Núñez, 2005; Butcher and Miles, 2002).

Species-specific trace metal concentration ranges are valuable tools for diagnosticians, researchers, and managers. Identification of metal toxicosis or deficiency requires either a substantial control group not often available in field studies or a comparison to some sort of "normal" reference range. Comparing hepatic metal concentrations from a domestic to a wild species is risky given the normal variation between species. If extrapolating from a domestic to a wildlife species, many host factors need to be taken into consideration. These include metal exposure, uptake, pharmacodynamics, pharmacokinetics, age, weight, sex, genetics, and nutritional status (Burger et al., 2003). Thus, species-specific reference ranges are a useful adjunct to tissue metals analysis.

In addition to simply providing baseline metals data for a wildlife species for which it is lacking, this study is important because Greater Sage-grouse are a species of concern, given their declining populations (Braun, 1998). Degradation and loss of suitable brood-rearing and nesting habitat, further complicated by the spread of WNv to western North America, has negatively affected Greater Sage-grouse populations (Naugle et al., 2004). While this species was denied federal protection under the Endangered Species Act (ESA) in January of 2005, efforts to conserve the species and their habitat continue (U.S. Fish and Wildlife Service, 2005). The baseline data generated in our study regarding this species will only aid in conservation efforts.

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