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SERUM CHEMISTRY VALUES FOR NESTLING BALD EAGLES (*HALIAEETUS LEUCOCEPHALUS*) IN FLORIDA BAY, EVERGLADES NATIONAL PARK

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Serum chemistry analysis involves the measurements of naturally-occurring enzymes and compounds, which are the result of metabolic and other physiological processes in the blood of birds (Harrison and Harrison 1986). The levels of several chemical compounds in the blood can be used in the diagnosis of the health status of individuals (Harrison and Harrison 1986).

More emphasis is being placed on the blood as a health-monitoring tool for wild birds (e.g., Dawson and Bortolotti 1997, Newman et al. 1997, Olsen et al. 2001, Balbontin and Ferrer 2002). Reference values are being established for plasma protein using electrophoresis as a diagnostic and prognostic means of assessing the health of several raptor species (Tatum et al. 2000). Establishing normal baseline values for the serum chemistries of free-ranging birds of prey will be important for future comparisons of population health. Interpreting serum chemistries must be done with scrutiny because age, sex, nutritional status and environmental conditions, circadian rhythms, and plasma and serum storage methods may influence these values (Ferrer 1993, Bustamante and Traviani 1993, Boal et al. 1998). Until more is known about serum chemistries and their applications as a monitoring tool for free-ranging raptors, these results should complement and be used with good ecological data for population assessments (Newman 1997).

Hematological analysis provides valuable information concerning the health status of an individual animal. For example, packed cell volume (PCV) is a good indicator of red blood cell mass (Howard and Matsumoto 1977), but age, sex (Boal et al. 1998), molt, reproductive activity, migration, dehydration, and diseases can affect avian PCVs (Carpenter 1975, Heidenreich 1997, Morishita et al. 1998). To date, there have been few clinical studies of serum chemistries of free-living birds. Normal chemistry values are extrapolated primarily from psittacines (parrots, macaws) and other domesticated fowl (poultry) living in captivity (Newman et al. 1997).

The objective of this cooperative study was to determine selected clinicopathologic parameters for nestlings of free-ranging Bald Eagle (*Haliaeetus leucocephalus*). Since knowledge of the basic physiology of these animals is limited, this protocol will contribute towards the establishment of 20 "normal" baseline hematological and serum values for Bald Eagle nestlings in Florida Bay, Everglades National Park, Florida, U.S.A.

METHODS

Study Site. The scope of this research involved monitoring multiple islands in Florida Bay, Everglades National Park (Fig. 1). The Florida Bay estuary (latitude 25°4′N and longitude 80°47′W) lies between the southern portion of the Florida peninsula to the north, the Florida Keys to the east and south and the Gulf of Mexico to the west. Nests were located using historical data from the South Florida Natural Resources Center of the Ever-

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Figure 1. The study site where the nestling Bald Eagles were sampled for serum chemistry between 1992 and 2001 was located in Florida Bay, Everglades National Park, Florida.

glades National Park or visually by following adults to the nest. The heights of the nests were ca. 1–10 m from the ground and built primarily on the black mangrove tree (*Avicennia germinans*). Surveys were conducted using an inflatable boat and fixed-wing aircraft. The keys on the eastern side of the bay had primarily flooded interiors, but the western keys of the bay were relatively vegetated and dry. The narrow shorelines of the eastern keys are spotted with sandy berms, which are fairly lush with dune plants including sea lavender (*Tournefortia gnaphalodes*), bay cedar (*Suriana maritima*), buttonwood (*Conocarpus erecta*), white mangroves (*Laguncularia racemosa*), bitter panic grass (*Panicum amarum*) and coast sandbur (*Cenchrus incertus*). The western keys were primarily fringed with the red mangrove (*Rhizophora mangle*), with the exception of Sandy Key, which had a sandy shoreline.

Aging and Sexing. Nests containing Bald Eagle nestlings 35–45 d old were visited from 1 January–1 May, from 1992–2001. Nestling development was monitored chronologically or through feather formation during repeated nest visits (Bortolotti 1984). No attempts were made to identify gender of nestlings because blood sampling occurred at 35–45 d. Sex determination using bill depth and foot length measurements may be applied when chicks are 60 d old (Bortolotti 1984). Clinical blood sexing techniques were not applied in this study. The nests were rarely monitored more than three times. Each nest was visited only once for blood sampling.

Blood Samples. When possible, blood was collected primarily in the mornings. Tides and weather played an extremely significant role when attempting to reach the islands. Circadian rhythms were considered prior to sampling, but occasionally the window within a 10-d period to reach an island was extremely narrow, and therefore, samples were taken opportunistically. Nestlings were hooded with a traditional falconry hood manufactured by Northwoods, Inc. (Rainier, WA) and removed from the nests by the investigators. Blood was extracted from the brachial vein (Cooper 1985, Hoysak and Weatherhead 1991). The area surrounding the vein was cleaned with 70% isopropyl alcohol and a sterile 22-, 23-, or 25-gauge needle attached to a 3-ml syringe was used to extract 1–3 ml of blood from each nestling. All blood extraction sites had pressure applied and were observed for approximately 5 min post procedure to ensure clotting prior to placing the nestling back into the nest (Hoysak and Weatherhead 1991). We returned to each nest ca. 7–10 d post sampling, when possible, to reevaluate the nestlings' condition.

Blood samples were placed in small 1-ml red top plastic microtainer tubes with a serum separator, (Becton Dickinson Co., Franklin Lakes, NJ) and heparinized hematocrit, made by Jorgensen Laboratories, Inc., (Loveland, CO), tubes and allowed to clot for 15 min prior to centrifugation. Samples were spun for ca. 20 min with a portable Mobilespin centrifuge (Vulcan Technologies, MO) with a relative centrifugal force of 1100 × *g*. Centrifuging was complete when the serum separator distinctly walled off the serum from the red blood cells. The serum was transferred with a pipette to another blood tube to prevent hemolysis. Field samples were placed in an ice cooler and later frozen. Samples were stored at −16.1°C and analyzed within 30 d.

Serum Samples. Eaglet sample sizes varied for each serum chemistry test depending on the amount of serum available. Thus, an adequate amount of serum was not available to measure all parameters, and sample sizes varied. One hematological, packed cell volume (PCV) and nineteen serum chemistries were determined including. total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), lactate dehydrogenase (LDH), creatine kinase (CK), uric acid (URIC), calcium (Ca^{2++}), phosphorus (PHOS), glucose (GLU), total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine (CRSC), sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), carbon dioxide (CO_2), and cholinesterase (CHE). Blood samples were analyzed on a Kodak Ektachem DT II System (Johnson and Johnson, Rochester, NY). Ektachem controls were run once a week to assure quality analysis control with all samples. Mean values and standard deviations were determined using JMP SAS (1994) statistical discovery software.

RESULTS AND DISCUSSION

We collected samples from 151 Bald Eagle chicks from 22 nests between 1992 and 2001. Our blood sampling studies did not have visible adverse affects on the health or fledging of the eaglets. The sampling area was devoid of markings and infections confirming the lack of any adverse effects from our blood sampling technique. Eaglet inactivity generally lasted for 20 min after sampling After that time eaglets sat up and started to call for food. The values for several serum parameters were variable, especially for LDH, CK, ALKP, AST, and URIC (Table 1).

Table 1. Hematological and serum chemistry values for Bald Eagle nestlings in Florida Bay, Everglades National Park.

MEASURE	*N*	MEAN ± SD	RANGE
PCV (%)	105	32.74 ± 4.16	17.0–42.0
GLU (mg/dl)	128	223.03 ± 27.37	150.0–292.0
PHOS (mg/dl)	151	6.31 ± 1.3	3.2–11.4
TP (g/dl)	125	3.28 ± 0.98	1.4–10.4
URIC (mg/dl)	147	13.46 ± 5.88	4.3–31.2
ALB (g/dl)	116	1.48 ± 0.43	1.0–3.2
ALKP (U/L)	123	147.57 ± 51.96	35.2–322.0
AST (U/L)	129	132.73 ± 48.04	46.0–357.0
ALT (U/L)	104	17.11 ± 7.41	3.0–42.0
Ca^{2++} (mg/dl)	148	9.39 ± 2.24	3.6–21.4
CHE (U/ml)	117	1.29 ± 0.31	0.3–2.5
CK (U/L)	117	1268.71 ± 557.69	473.2–3190.0
LDH (U/L)	79	2547.63 ± 965.22	1045.0–7479.0
CRSC (mg/dl)	42	0.24 ± 0.11	0.1–0.5
TBIL (mg/dl)	59	1.24 ± 1.06	0.3–4.1
BUN (mg/dl)	75	13.41 ± 10.79	2.0–44.0
Na^+ (nmol/L)	135	135.85 ± 11.13	99.0–164.0
Cl^- (mmol/L)	136	104.98 ± 10.11	67.0–127.0
K^+ (mmol/L)	132	4.28 ± 1.82	2.2–9.7
CO_2 (mmol/L)	117	18.9 ± 4.79	6.0–34.0

Currently the interpretation of avian biochemistries is difficult due to the lack of controlled studies and available references. Raptor biologists and wildlife veterinarians must also be aware that values between adults, juveniles, and nestlings may vary significantly (Boal et al. 1998). Collected data provide baseline parameters of free-ranging Bald Eagle nestlings in Florida Bay (Table 1) Even though raptor nestling serum values are not well-documented in the literature, the results of this study appear to be within the reported parameters for other free-ranging raptor nestlings.

The normal PCV ranges for parrots (Psittacidae) and other domestic birds is 37–53% (Harrison and Harrison 1986), seabirds 39–45% (Newman 1997, Work 1999), and for captive adult Bald Eagles 35–50% (Ivins et al. 1978). In other studies of free-ranging raptors, PCV are very variable (Balasch et. al. 1976, Hunter and Powers 1980). This variation may be due to age and sex, migrating status, and reproduction (Boal et al. 1998, Stein et al. 1998). Our results showed that PCV mean values for the eaglets of 32.74% (Table 1) in this study fall within the range (31–38%) of free-ranging nestling eagles in Chippewa National Forest (CNF) (Redig et al. 1983) and nestlings in the lower peninsula of Michigan (Bowerman et al. 2000). In contrast, results for PCV mean values for free-ranging nestling Cooper's Hawks (*Accipiter cooperii*) was 42.2% for females and 38.7% for males (Boal et al. 1998).

Most normal avian values for total protein range between 3.0 and 5.0 g/dl. Values that fall below 2.5 g/dl may reflect parasitism, stress, or starvation. Values greater than 5 g/dl may indicate dehydration, shock, or infection (Harrison and Harrison 1986). TP values in this study ranged 1.4–10.4 g/dl with a higher variation than those in Michigan (Bowerman et al. 2000). The mean values for TP in Florida Bay 3.28 g/dl were similar with the study sites in Michigan at 3.4 g/dl, but lower than results from CNF at 4.7 g/dl (Redig et al. 1983).

Uric mean and range values for Bald Eagle nestlings in Florida Bay were higher than the Bonelli's Eagle (*Hieraaetus fasciatus*) nestling mean values of 7.77 mg/dl (684.2 umol L^{-1}) for males and 9.4 mg/dl (827 umol L^{-1}) for females (Balbontin and Ferrer 2002). High levels of uric acid in free-ranging eagle nestlings may be attributed to food stress (Ferrer 1994). GLU mean levels in eaglets in Florida Bay are lower than those in Michigan (280 mg/dl), but both fall within the normal range of 200–500 mg/dl for captive birds. Electrolytes results for Na^+, K^+, and Cl^- in Florida Bay were within the ranges of other Bald Eagle nestling studies (Redig et al. 1983, Bowerman et al. 2000). There were variations when compared with the Bonelli's Eagle (Balbontin and Ferrer 2002) and below the values of several European raptors (Polo et al. 1992, Jenkins 1994, Stein 1998).

High variation of CK and LDH levels in this study may be due to the handling and physiology of nestling eagles and is in accord with the results found in free-living Bonelli's Eagle, Booted Eagles (*Hieraaetus pennatus*), and the Spanish Imperial Eagles (*Aquila adalberti*) (Polo et al. 1992, Balbontin and Ferrer 2002, Casado et al. 2002).

CHE levels <0.9 u/ml are considered depressed and

likely due to intoxications (Porter 1993, Heatley and Jowett 2000). In 1997, one eaglet from Frank Key and one from Park Key were found to have depressed levels of CHE. Both eaglets fledged successfully. It is possible that these eaglets may have been exposed to organophosphates after consuming avian prey brought in by the parents. In contrast, Osprey (*Pandion haliaetus*) had CHE levels above 1.0 u/ml (B. Mealey unpubl. data). There is some question about the value of using CHE as an indicator for exposure to an organophosphate pesticide due to the variations in CHE levels over a short period of time (P. Mineau pers. comm.).

Reference values for PHOS in domestic birds are 2–6 mg/dl. The mean values of eaglets in Florida Bay (Table 1) were in the range of other Bald Eagle nestling studies (Redig et al. 1983, Bowerman et al. 2000) and similar to the mean results for adult Spanish Imperial Eagles (5.8 mg/dl), Golden Eagles (*Aquila chrysaetos*; 4.7 mg/dl), Griffin Vultures (*Gyps fulvus*; 4.3 mg/dl), and Egyptian Vultures (*Neophron percnopterus*; 7.3 mg/dl) (Polo et al. 1992, Dobado-Berrios et al. 1998).

ALKP mean results for free-ranging eagle nestlings in Florida Bay (147.5 U/L) were similar to adult eagles and lower than found by Bowerman (2000) in Michigan (449 U/L) and Balbontin (2002) in Spain (2148 UI L^{-1}).

Evaluation of serum chemistries offers wildlife biologists and federal and state agencies an additional way of monitoring the health of free-ranging raptor populations. If there is an opportunity to gather additional data on blood serum parameters from a listed raptor population, then every effort should be made to enhance the existing pool of knowledge concerning such species. As with every technique, precautions should be taken to avoid any potential injury or undue stress to the animals.

Data interpretation from the serum chemistries is subject to debate because of the many variables that affect particular enzymes. Standardized protocols should be established and long-term studies on the baseline values of a particular species should be conducted. Areas in need of attention and further research with free-living raptors are: (1) the effects of capture and handling and how this stress may affect results; (2) how does age, sex, and season alter the serum values; and (3) the effects of nutrition on these serum value indicators. Evaluations of serum chemistries are in its infancy stage for free-ranging birds of prey. As further research is conducted, the information gathered will aid in the applications of serum chemistry analysis as a tool for the management of raptor populations.

RESUMEN.—El análisis de la química del suero esta comenzando a ser una herramienta de diagnostico vital para evaluar la salud de las aves de presa en libertad. La química del suero de 151 polluelos de águilas calvas (*Haliaeetus leucocephalus*) en vida silvestre, fue medida de 1992—2001 en la bahía de la Florida, Parque Nacional Everglades. Medimos 19 valores de suero y un valor hematológico (PCV) para establecer los parámetros normales de línea base para los aguiluchos. Los resultados de los valores de suero fueron consistentes con los resultados de otras poblaciones de aves rapaces en libertad.

[Traducción de César Márquez]

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