

PLASMA CHEMISTRY REFERENCE VALUES IN FREE-LIVING BONELLI'S EAGLE  
(*HIERAAETUS FASCIATUS*) NESTLINGS

JAVIER BALBONTÍN<sup>1</sup> AND MIGUEL FERRER

Department of Applied Biology, Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas,  
Avda de Maria Luisa s/n, Pabellón del Perú, Sevilla 41013 Spain

KEY WORDS: *Bonelli's Eagle*, *Hieraaetus fasciatus*; blood; free-living nestling; plasma chemistry; sex.

Few studies dealing with plasma biochemistry and physiology in wild birds can be found in the scientific literature. Most research papers deal with domestic or captive birds kept in zoos, rehabilitation facilities, or research centers (Lewandoski et al. 1986, Redig 1991, Ferrer 1993, Dobado-Berrios et al. 1998). The knowledge of normal reference values in plasma for wild species is very important to a wide range of multidisciplinary subjects. Veterinarians need to have this information in order to better diagnose the condition of wild birds entering into rehabilitation centers or zoos (Lepoutre et al. 1983, Cooper et al. 1986). Also, information gathered from hematological research is of a great importance for ecologists because such data may provide insights into the health of individuals being studied. Body condition that is related to other ecological factors such as survival, fecundity, or habitat quality, could be estimated by levels of urea, uric acid, and other blood parameters (Cherel et al. 1987, Ferrer et al. 1987, García-Rodríguez et al. 1987, Robin et al. 1987).

It is important to know normal blood parameter reference values for endangered species involved in a reintroduction or restoration program in order to better understand the physiological status of the released birds. Normal reference values in blood chemical constituents are known only for 5% of bird species which have been studied mostly in captive situations (Ferrer 1993).

Although most of the information available comes from captive birds, it might be expected that a captive condition might affect hematological values (Bell and Freeman, 1971, Miglirioni et al. 1973, Wolf et al. 1985, Sturkie 1986, Ferrer et al. 1987, García-Rodríguez et al. 1987). Factors such as age or sex influence the total variation found in plasma enzymes, proteins, metabolites, and other organic molecules. However, presently these factors are poorly understood due to difficulty of gathering information on different age-classes in wild species. Other factors affecting values of chemical components in plasma are circadian rhythm (García-Rodríguez et al. 1987), seasonal changes (Wolf et al. 1985), or plasma storing methods (Bustamante and Traviani 1993).

The Bonelli's Eagle (*Hieraaetus fasciatus*) is an endan-

gered species that has suffered a rapid population decline in most areas of Europe including Spain (Cugnasse 1984, Palma et al. 1984, Hallmann 1985, Arroyo et al. 1990). In this article, we present normal chemical plasma values found in a free-living endangered population of nestlings of this bird of prey. Data from 21 biochemical substances (including metabolites, total protein, inorganic ions, and enzyme activities) and differences found between sexes in this age-class are reported. In addition, we have examined the differences found between free-living and captive birds of this long-lived raptor.

METHODS

We have studied a breeding population of Bonelli's Eagles in the province of Cádiz that is located in southern Spain (5°32'W, 36°41'N). We collected blood from both nestlings of a free-living population of south Spain and from captive young Bonelli's Eagles. The diet of eagles in our region included a preponderance of rabbits (*Oryctolagus cuniculus*), and Red-legged Partridge (*Alectoris rufa*) (Gil-Sánchez et al. 1994, Ontiveros and Pleguezuelos 2000). Birds kept in captivity were fed ad libitum with partridge and rabbit. Blood collected from free-living nestlings was taken when individuals were between 47–53-d-old, about 10 d before fledging. One of us climbed or descended to several nests each year to band and measure young; at the same time 2 ml of blood was extracted from the brachial vein of the wing. To minimize circadian variations of blood parameters, we extracted all blood samples between 1100–1500 H. CST blood was collected in lithium-heparin tubes and the plasma was separated by centrifugation (10 min: 907.2 × g). Cellular fraction and plasma samples were immediately frozen (–80°C). Analyses were carried out 4 mo later with a Hitachi 747 multichannel automatic analyzer (Tokyo, Japan) with the reagents recommended by Boehringer-Mannheim (Darmstadt, F.R.G.). Plasma was analyzed (abbreviations and methods indicated in parentheses) for amylase (AMY; maltoheptaose reaction), cholesterol (CHOL; cholesterol esterase), creatinine (CREA; Kinetic Jaffé reaction), creatinine kinase (CK; optimal standard method DGKC), glucose (GLUC; hexokinase method), aspartate aminotransferase (AST; DGKC technique), alanine aminotransferase (ALT; DGKC technique), total protein (TP; biuret reaction), triglycerides (TRIG; enzymatic method), urea (UREA; urease method, uric acid (UA, uricase method), alkaline phosphatase (AP; paranitrophenylphosphate method), colinesterase (CHE), L-lactate dehydrogenase (LDH; SFBC technique), bilirubin (BILIR; DPD method), calcium (Ca; cresolphthalein complexone reaction), phosphorus (iP; molybdenum blue reaction),

<sup>1</sup> E-mail address: Balbonja@ebd.csic.es

sodium (Na; Indirect potentiation, E. Selective), potassium (K; Indirect potentiation, E. Selective), magnesium (Mg; blue xilidil reaction).

The cellular fraction of the blood sample was used to sex all eagles. For this analysis, primers 2945F, cR and 3224R were used following Ellegren (1996). The total number of eagles sampled included 28 nestlings, 14 females and 14 males, belonging to a free-living population and two young males and three young females that were captive birds.

We used the Student's *t*-test to check for differences in the means of blood parameters between sexes and between captive and free-living birds. Non-parametric tests were employed for those variables not meeting either normality or homoscedasticity assumptions (Siegel and Castellan 1988). When data were not normally distributed, they were  $\log_e$  transformed (Sokal and Rohlf 1995). After this transformation only two blood parameters AP and K, did not exhibit a normal distribution. The assumption of homoscedasticity was met for those parameters showing a normal-like distribution. Levene's *F* test was used to test for homoscedasticity. All tests were two-tailed and statistical significance was set at  $P < 0.05$ . Means are given with  $\pm$ SD. Range for all variables are also provided. In some cases due to the small plasma volume of some samples, it was not possible to do analyses of all chemical parameters, thus, the sample size is not the same for all blood components. Analyses were performed with the Statistica Ax 99 package (Statistica, 1996. Version 5. Statsoft, Inc.).

## RESULTS

Normal reference plasma chemistry values for nestling Bonelli's Eagles are shown in Table 1. We found differences in parameters between male and female free-living nestlings in two of 21 plasma parameters measured. Males showed higher glucose levels in blood and lower AST activity than females (Table 1). We found that urea ( $t = 3.78$ ,  $df = 32$ ,  $P < 0.001$ ), uric acid ( $t = 3.21$ ,  $df = 33$ ,  $P < 0.001$ ), alkaline phosphatase ( $t = 3.52$ ,  $df = 32$ ,  $P < 0.001$ ) and creatinine kinase ( $t = 2.5$ ,  $df = 32$ ,  $P < 0.05$ ) values were lower than these measured in captive eagles. However, glucose ( $t = -3.89$ ,  $df = 33$ ,  $P < 0.001$ ) was higher in captive compared with free-living birds.

## DISCUSSION

Our data showed that there were differences in plasma glucose levels between sexes in nestling Bonelli's Eagles, males showing higher levels than females (Table 1). Polo (1995) failed to find any difference in this parameter between sexes in eight avian orders including Falconiformes. However, Polo (1995) only examined birds that were in captivity. Levels of glucose in plasma have been correlated with metabolic rate (Umminger 1977). Birds that showed a high metabolic rate, because of high activity such as flying with fast flapping, would also have higher levels of glucose in plasma. Bonelli's Eagle exhibits a strong sexual size dimorphism, with females being much larger than males, which implies that male and female nestlings of this species would have different metabolic

rates due to different growth patterns. Consequently, each sex may be exposed to a different energy demand during the nestling period. For example, female nestlings gain an estimated 7.3 grams more than males each day during the nestling period, which lasts on average between 59 (Minguez et al. 2001) and 63 d (Real et al. 1998). Therefore, males might have higher glucose levels because they have a lower growth energy demand than females. The larger females might channel more glucose into tissue formation than smaller males. Recently, Casado et al. (2002) also found that male nestling Booted Eagles (*Hieraetus pennatus*) had higher glucose than females, another raptor with strong sexual size dimorphism. However, González and Hiraldo (1991) studying free-living nestling Marsh Harriers (*Circus aeruginosus*) (also shows sexual size dimorphism), found the reverse tendency with female nestlings having higher glucose levels than males. A possible explanation is that glucose level in nestlings could indicate the quantity and quality of food received by each individual during the growing period. Parents might be able to allocate food in an asymmetric way within brood favoring either sex depending on environmental condition or food availability; for example, the larger or older sibling may receive more food in years of scarcity (Mock et al. 1987).

As most of the knowledge in normal plasma reference values comes from captive birds, we compared blood chemistry values from our free-living nestling sample with values gathered from five juvenile captive eagles. These birds were young eagles in their first year; therefore, they were a few months older (less than a year different) than eagles sampled in nature. For this reason, age may have affected the differences found between these two groups. Among the differences we found were those related to nitrogen residues. Captive birds had lower UREA and UA than free-living ones. These chemical constituents have been associated with physical condition in birds of prey. An increase in plasma levels of these nitrogen residues has been predicted when birds are subjected to a food stress situation (Ferrer 1994, Alonso-Alvarez and Ferrer 2001). Therefore, lower levels in UREA and UA in captive birds indicate that these eagles were in better body condition than free-living birds.

In this study we also found that captive eagles showed higher plasma glucose levels than free-living ones, which might be in accordance to what has been reported in other birds (Lewandoski et al. 1986, Casado et al. 2002). Captive individuals also showed lower CK activity than free-living ones. This enzyme mediates in muscle contraction and is related with physical activity. Thus, it seems reasonable that eagles in captivity would show lower CK activity than free-living eagles. The difference found on AP activity between captive and free-living birds might be attributed to an age effect rather than to a captive condition since it is well known that the activity of this enzyme decreased with aged in birds of prey. Concretely, this enzyme is related to the ossification of frontal

Table 1. Blood chemistry values for male and female nestling Bonelli's Eagles from Cádiz, Spain.

VARIABLE	(SI UNIT)	MALES			FEMALES			N	t	df	P <sup>a</sup>
		MEAN ± SD	RANGE	N	MEAN ± SD	RANGE	N				
GLU	(mmol L <sup>-1</sup> )	15.74 ± 2.09	(12.06–18.50)	14	14.03 ± 1.39	(11.3–15.7)	14	2.53	26	0.017*	
UREA	(mmol L <sup>-1</sup> )	2.27 ± 0.56	(1.33–3.17)	13	2.52 ± 0.98	(0.92–4.33)	14	-0.84	25	0.40	
UA	(μmol L <sup>-1</sup> )	684.2 ± 279.6	(434.3–1362.5)	14	827.0 ± 307.6	(321.3–1362.5)	14	-1.51	26	0.14	
CREAT	(μmol L <sup>-1</sup> )	24.8 ± 4.43	(17.7–35.4)	14	25.6 ± 3.54	(21.2–32.7)	14	-0.44	26	0.66	
PT	(g/l)	29.8 ± 1.5	(26–32)	10	30.1 ± 2.8	(27–36)	10	-0.36	18	0.71	
AP	(UI L <sup>-1</sup> )	2148 ± 696	(509–3160)	13	2280 ± 327	(1696–2759)	14	-0.63 (1)	25	0.52 <sup>b</sup>	
CHE	(UI L <sup>-1</sup> )	1191 ± 272	(961–1860)	9	1140 ± 228	(789–1571)	10	0.45	18	0.65	
AMY	(UI L <sup>-1</sup> )	1148 ± 303	(784–1776)	9	942 ± 194	(726–1310)	12	1.90	19	0.07	
CK	(UI L <sup>-1</sup> )	3859 ± 883	(2116–5034)	13	3853 ± 918	(1880–5352)	14	0.01	25	0.98	
LDH	(UI L <sup>-1</sup> )	1647 ± 160	(1489–1903)	6	1828 ± 355	(1199–2417)	8	-1.15	12	0.27	
COL	(mmol L <sup>-1</sup> )	4.35 ± 0.60	(3.06–5.54)	16	4.53 ± 0.73	(3.29–5.80)	14	-0.65	26	0.51	
TG	(mg/dl)	71.3 ± 35.1	(28–166)	13	81.7 ± 36.6	(36–161)	14	-0.93	25	0.35	
MG	(mmol L <sup>-1</sup> )	0.77 ± 0.04	(0.54–0.70)	6	0.72 ± 0.07	(0.54–0.79)	8	-1.67	12	0.12	
NA	(mmol L <sup>-1</sup> )	66.09 ± 1.27	(65–67.7)	5	65.95 ± 1.81	(62.2–68.1)	8	0.13	11	0.89	
K	(mmol L <sup>-1</sup> )	22.3 ± 11.9	(0.89–28.1)	5	21.2 ± 11.7	(1.15–28.6)	8	0.21 (1)	11	0.82 <sup>b</sup>	
CA	(mmol L <sup>-1</sup> )	2.15 ± 0.25	(1.63–2.38)	6	2.20 ± 0.23	(1.80–2.45)	8	-0.25	12	0.80	
P	(mmol L <sup>-1</sup> )	1.55 ± 0.23	(1.2–1.94)	7	1.49 ± 0.19	(1.10–1.68)	8	0.54	13	0.59	
BILIR	(μmol L <sup>-1</sup> )	1.19 ± 0.17	(0.85–1.70)	6	1.02 ± 0.51	(0.51–2.22)	8	0.44	12	0.66	
AST	(UI L <sup>-1</sup> )	171.6 ± 18.2	(153–202)	6	203.2 ± 31.5	(139–241)	8	2.17	12	0.04*	
ALT	(UI L <sup>-1</sup> )	11.5 ± 2.2	(10–16)	6	13.3 ± 3.8	(9–19)	8	-1.04	12	0.31	
GGT	(UI L <sup>-1</sup> )	14.5 ± 14.5	(7–53)	9	15.1 ± 10.0	(5–39)	12	-0.41	19	0.67	

<sup>a</sup> Probability that means are equal calculated with a Student's *t*-test.

<sup>b</sup> Probability that distributions are equal calculated with a Mann-Whitney *U*-test.

\* Significantly different, *P* < 0.05.

bones that take place throughout the immature to adult age period (Dobado-Berrios and Ferrer 1997, Viñuela et al. 1991).

RESUMEN.—Este artículo presenta información sobre los valores de referencia normales de parámetros bioquímicos presente en plasma sanguíneo de pollos estudiados en libertad de Águila perdicera (*Hieraaetus fasciatus*). Investigamos diferencias en los parámetros sanguíneos entre sexos. Los machos muestran unos niveles más altos de glucosa y una actividad enzimática de Alanino-amino transferasa (AST) mayor que las hembras. Los pollos fueron comparados con jóvenes águilas mantenidas en cautividad. Las águilas cautivas tuvieron valores más bajos de urea, ácido úrico, fosfatasa alcalina, creatinina quinasa y valores más altos de glucosa que los pollos marcados en libertad.

[Traducción de los autores]

#### ACKNOWLEDGMENTS

We want to acknowledge the help of E. Minguez, J.R. Benitez, V. Siebering, C. Aguilar, J.A. Gil, and E. Saez in the field. We also thank E. Calvo for climbing to cliff nests. C.F. Vega and C. Alonso helped with blood analysis. F. Recio and the lab personnel of Valme Hospital allowed us to use their lab equipment. Z. Arhzaf gave us access to captive birds. This study was supported by the regional government (Junta de Andalucía, Consejería de Medio Ambiente).

#### LITERATURE CITED

- ALONSO-ALVAREZ, C. AND M. FERRER. 2001. A biochemical study of fasting, subfeeding, and recovery processes in Yellow-legged Gulls. *Physiol. Biochem. Zool.* 74:703–713.
- ARROYO, B., E. FERREIRO, AND V. GARZA. 1990. Inventario de la población Española de Águila Perdicera (*Hieraaetus fasciatus*) y sus áreas de cría. ICONA, Madrid, Spain.
- BELL, D.J. AND D.M. FREEMAN. 1971. Physiology and biochemistry of the domestic fowl. Vol. 2. Academic Press, New York, NY U.S.A.
- BUSTAMANTE, J. AND A. TRAVIANI. 1993. Effect of keeping plasma frozen at  $-20^{\circ}\text{C}$  on the concentration of blood metabolites. *Comp. Biochem. Physiol.* 106:661–664.
- BRUGÈRE-PICOUX J., H. BRUGERE, L. BASSET, N. SAYAD, J. VAAST, AND J.M. MICHAUX. 1987. Biochemie Clinique en pathologie aviaire. Intérêt et limites des dosages enzymatiques chez la paule. *Recl. Méd. Vét.* 163:1091–1099.
- CASADO, E., J. BALBONTÍN, AND M. FERRER. 2002. Plasma chemistry in Booted Eagle (*Hieraaetus pennatus*) during breeding season. *Comp. Biochem. Physiol.* 131:233–241.
- CHEREL, Y., J.C. STAHL, AND Y. LE MAHO. 1987. Ecology and physiology of fasting in King Penguin chicks. *Auk* 104:254–262.
- COOPER, J.E., J.R. NEEDHAM, AND N.C. FOX. 1986. Bacteriological, haematological and clinical chemical studies in the Mauritius Kestrel (*Falco punctatus*). *Avian Pathol.* 15:349–356.
- CUGNASSE, J.M. 1984. L'Aigle de Bonelli *Hieraaetus fasciatus* en Languedoc-Roussillon. *Nos Oiseaux* 37:223–232.
- DOBADO-BERRIOS, P. AND M. FERRER, M. 1997. Age-related changes of plasma alkaline phosphatase and inorganic phosphorus, and late ossification of the cranial roof in the Spanish Imperial Eagle (*Aquila adalberti* C.L. Brehm, 1861). *Physiol. Zool.* 70:421–427.
- , J.L. TELLA, O. CEBALLOS, AND J.A. DONÁZAR. 1998. Effects of age and captivity on plasma chemistry values of the Egyptian Vulture. *Condor* 100:719–725.
- ELLEGREN, H. 1996. First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. *Proc. R. Soc. Lond.* 263:1635–1641.
- FERRER, M. 1993. Blood chemistry studies in birds: some applications to ecological problems. Pages 1031–1044 in S.G. Pandali [Ed.], Trends in comparative biochemistry and physiology. Council Scientific Research Integration, Trivandrum, India.
- . 1994. Nutritional condition of Spanish Imperial Eagle nestling (*Aquila*). *Bird Study* 41:120–123.
- , T. GARCÍA-RODRIGUEZ, J.C. CARRILLO, AND J. CASTROVIEJO. 1987. Hematocrit and blood chemistry values in captive raptors (*Cyps fulvus*, *Buteo buteo*, *Milvus migrans*, *Aquila heliaca*). *Comp. Biochem. Physiol.* 87:1123–1127.
- GARCÍA-RODRIGUEZ, T., M. FERRER, F. RECIO, AND J. CASTROVIEJO. 1987. Circadian rhythms of determined blood chemistry values in Buzzards and Eagle Owls. *Comp. Biochem. Physiol.* 88:663–669.
- GIL-SÁNCHEZ, J.M., F.M. MOLINO, AND G. VALENZUELA. 1994. Parámetros reproductivos y alimentación del Águila Real (*Aquila chrysaetos*) y del Águila Perdicera (*Hieraaetus fasciatus*) en la Provincia de Granada. *Aegypius* 12:47–51.
- GONZÁLEZ, J.L. AND F. HIRALDO. 1991. Some hematological data from Marsh Harriers *Circus aeruginosus* in central Spain. *Comp. Biochem. Physiol.* 100:735–737.
- HALLMANN, B. 1985. Status and conservation of birds of prey in Greece. Pages 55–59 in Newton, I. and R.D. Chancellor [Eds.], Conservation studies on raptors, ICBP Technical Publication, London, U.K.
- LEPOUTRE, D., J. BARBAT, M. GUITET, AND J. BLANCOU. 1983. Contribution a l'étude de la biologie de quelques rapaces en France. *Rev. Méd. Vét.* 134:481–493.
- LEWANDOSKI, A.H., T.W. CAMPBELL, AND G.J. HARRISON. 1986. Clinical chemistries. Pages 192–200 in G.J. Harrison and L.R. Harrison [Eds.], Clinical avian medicine and surgery. W.B. Saunders Co. Philadelphia, PA U.S.A.
- MIGLIORINI, R.H., C. LINDER, J.L. MOURA, AND J.A. VEIGA. 1973. Gluconeogenesis in a carnivorous bird (Black Vulture). *Am. J. Physiol.* 225:1389–1392.
- MINGUEZ, E., E. ANGULO, AND V. SIEBERING. 2001. Factors

- influencing length of the post-fledging period and timing of dispersal in Bonelli's Eagle (*Hieraetus fasciatus*) in southwestern Spain. *J. Raptor Res.* 35:228–234.
- MOCK, D.W., T.C. LAMEY, AND B.J. PLOGER. 1987. Proximate and ultimate roles of food amount in regulating egret sibling aggression. *Ecology* 35:1760–1772.
- ONTIVEROS, D. AND J.M. PLEGUEZUELOS. 2000. Influence of prey densities in the distribution and breeding success of Bonelli's Eagle (*Hieraetus fasciatus*): management implications. *Biol. Conserv.* 93:19–25.
- PALMA, L., F. CANCELADA, AND L. OLIVEIRA. 1984. L'alimentation de l'Aigle de Bonelli (*Hieraetus fasciatus*) dans la côte Portugaise. *Rapinyaires Mediter.* 2:87–96.
- PERRY, M.C., N.H. OBRECHT, B.K. WILLIAMS, AND W.J. KUENZEL. 1986. Blood chemistry and hematocrit of captive and wild canvasback. *J. Wildl. Manage.* 50:435–441.
- POLO, F.J. 1995. Estudio bioquímico y enzimático del plasma de aves en cautividad. Ph.D. dissertation. Universidad de Barcelona, Barcelona, Spain.
- REAL, J., S. MAÑOSA, AND J. CODINA. 1998. Post-nestling dependence period in the Bonelli's Eagle *Hieraetus fasciatus*. *Ornis Fenn.* 75:129–137.
- REDIG, P. 1991. Medical management of birds of prey. The Raptor Center. University of Minnesota, St. Paul, MN U.S.A.
- ROBIN, J.P., Y. CHEREL, H. GIRARD, A. GÉLGEN, AND Y. LE MAHO. 1987. *J. Comp. Physiol.* 157:491–499.
- ROSSKOPF, W.J., R.W. WOERPEL, G.A. ROSSKOPF, AND D. VAN DE WATER. 1982. Haematological and blood chemistry values for commonly kept cockatoos. *Calif Vet.* 36:11–13.
- SIEGEL, S. AND N.J. CASTELLAN. 1988. Nonparametric statistics for the behavioural sciences. McGraw-Hill, New York, NY U.S.A.
- SOKAL, R.R. AND F.J. ROHLF. 1995. Biometry, the principles and practice of statistics in biological research, 3rd Ed. W.H. Freeman, New York, NY U.S.A.
- STURKIE, P.D. 1986. Avian physiology. Springer-Verlag, New York, NY U.S.A.
- UMMINGER, B.L. 1977. Relation of whole blood sugar concentration in vertebrates to standard metabolic rate. *Comp. Biochem. Physiol.* 56:457–460.
- VIÑUELA, J., M. FERRER, AND F. RECIO. 1991. Age-related variations in plasma levels of alkaline phosphatase Calcium and inorganic phosphorus in chicks of two species of raptors. *Comp. Biochem. Physiol.* 99:49–54.
- WOLF, S.H., R.W. SCHREIBER, L. KAHANA, AND J.J. TORRES. 1985. Seasonal, sexual and age-related variation in the blood composition of the Brown Pelican (*Pelicanus occidentalis*). *Comp. Biochem. Physiol.* 82:837–846.

Received 31 December 2001; accepted 23 May 2002