ORNITOLOGÍA NEOTROPICAL

(2023) 34: 24-28

DOI: 10.58843/ornneo.v34i1.1061



SHORT NOTE

HEMATOLOGICAL AND SERUM CHEMISTRY REFERENCE VALUES OF CAPTIVE BLACK-CHESTED BUZZARD-EAGLES GERANOAETUS MELANOLEUCUS

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Abstract • 30 captive Black-chested Buzzard-Eagles *Geranoaetus melanoleucus* from the Raptors Rehabilitation Center of the Union of Ornithologists of Chile were used to determine blood reference values. Packed cell volume (PCV), total protein plasma (TPP), and total and differential leukocyte counts were measured. Gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine kinase (CK), creatinine (CRE), calcium (Ca), inorganic phosphorus (P) and uric acid (UA) were analyzed. No significant differences were found between sexes (P> 0.05). No blood parasites were found. The results obtained from this study establish a baseline for hematological and serum biochemistry parameters for the Black-chested Buzzard-Eagles.

Resumen · Valores de referencia para parámetros hematológicos y bioquímicos de águilas moras Geranoaetus melanoleucus en cautiverio.

En este estudio, 30 águilas mora *Geranoaetus melanoleucus*, mantenidas en cautiverio en el Centro de Rehabilitación de Rapaces de la Unión de Ornitólogos de Chile, fueron utilizadas para la determinación de valores de referencia. Se midieron el volumen de células plasmáticas (PCV), la proteína plasmática total (TPP) y recuentos totales y diferenciales de leucocitos. Los niveles de Gamma-glutamil transpeptidasa (GGT), lactato deshidrogenasa (LDH), fosfatasa alcalina (FA), creatina quinasa (CK), creatinina (CRE), calcio (Ca), fósforo inorgánico (P) y ácido úrico (AU) fueron analizados. No se encontraron diferencias significativas entre sexos (P> 0.05). No se encontraron parásitos sanguíneos. Los resultados obtenidos de este estudio establecen una línea base para los parámetros hematológicos y bioquímicos del suero del Águila mora.

Key words: Biochemistry · Hematology · Raptors

Reference values for hematological and biochemical parameters of wild birds are important for veterinarians dealing with captive and rehabilitating birds. These reference values can be useful in determining the health or general condition of birds (Nazifi et al. 2008). Data on hematological and biochemical values for the Black-chested Buzzard-Eagle *Geranoaetus melanoleucus* are not currently available despite the species ranging throughout South America (Couve & Vidal 2000).

In January 2015, blood samples were collected from 30 captive Black-chested Buzzard-Eagles (22 adults and 8 juveniles; 22 females and 8 males), from the Rehabilitation Center for Raptors of the Union of Ornithologists of Chile, city of Talagante (33°40'00''S, 70°56'00''W). Samples were collected immediately prior to release, or in birds intended for permanent captivity, when the birds were considered to be in normal and healthy condition. A veterinarian physically evaluated all birds included in this study and considered all to be clinically healthy. Age and sex of each bird were determined by plumage (Martínez & González 2005). In this study, conditions such as housing, diet, and veterinary management were the same for all individuals sampled.



We collected 2 ml from the metatarsal vein using sterile syringes with 21-gauge needles and transferred them to Vacutainer[®] tubes with ethylenediaminetetraacetic acid (EDTA) as anticoagulant and Vacutainer[®] tubes without anticoagulant for haematology and biochemistry analyses, respectively. Blood samples were processed in the Clinical Laboratory of the School of Veterinary Sciences at the Universidad de Concepción. Haematological and biochemical analyses were performed within 12 hours of sample collection.

Packed cell volume (PCV; %) was measured from the microhematocrit tubes; these microhematocrit tubes were filled with the blood from EDTA tube after centrifuging for 4 min at 14,490 g in a microhematocrit centrifuge (Campbell & Coles 1986; Digital microcentrifuge, KHT 410E. Taipei, Taiwan, Republic of China). Total plasma protein (TPP; g/L) was determined by Goldberg's refractometer. White blood cell count (WBC; x10⁹/L) was determined by Neubauer chamber, using a modified version of the solution described by Rees and Ecker as a solvent (Lucas & Jamroz 1961).

Blood smears were stained with Diff-Quick[®]. Differential white blood cell counts were performed by counting 200 leukocytes using oil immersion microscopy at 1,000X magnification (Merino et al. 1997). Blood smears were examined for blood parasites using oil immersion microscopy at 1,000x magnification.

Serum and cells were separated by centrifuging at 2,500 g for 10 min (Centrifuge DSC 200A-2 Already Enterprise Inc.

Taipei, Taiwan, Republic of China), and the obtained serum was processed for blood chemistry determinations. Gammaglutamyl transpeptidase (GGT; U/L), lactate dehydrogenase (LDH; U/L), alkaline phosphatase (ALP; U/L), creatine kinase (CK; U/L), creatinine (CRE; µmol/L), calcium (Ca; mmol/L), inorganic phosphorus (P; mmol/L) and uric acid (UA; µmol/L) were determined using a commercial test (DiaSys Diagnostic Systems GmbH, Alte Strasse 9, Holzheim, Germany). The LDH and CK were read at 340 nm, GGT and ALP at 405 nm, CRE at 546 nm, Ca at 570 nm, and UA at 520 nm in a Spectrophotometer (Wiener lab, MetroLAB 2300 Plus UV-Vis Metrolab S.A., Buenos Aires, Argentina). We tested normality with the Anderson-Darling test (RefValAdv, National Veterinary School, Toulouse, France). Some variables were not normally distributed, and data were transformed to approximate a normal distribution using the Box-Cox transformation (Gotelli and Ellison 2004). We determined reference intervals and 90% confidence intervals of the limits using the Freeware Reference Values Advisor (RefValAdv, National Veterinary School, Toulouse, France) macroinstructions for Excel®. All statistical findings were considered significant at P≤0.05. Data are expressed as mean ± standard deviation (SD) and reference intervals. Because no information is available on the parameters for hematological and biochemical data of Black-chested Buzzard-Eagles, the values were compared with those of other raptors.

Hematological and biochemistry data are presented in tables 1-4. Significant statistical differences between adults and

 Table 1. Hematological parameters of rehabilitated adult and juvenile Black-chested Buzzard-Eagles Geranoaetus melanoleucus housed in the Rehabilitation

 Center.

ANALYTE		N	MEAN	SD	MEDIAN	MIN	MAX	RI	L REF LIM 90% CI	U REF LIM 90% CI	DIST
PCV %	Total	29	42.0	4.1	42.0	35.0	50.0	33.4-50.5 °	31.4-35.6	48.2-52.6	Normal
	Juvenile	7	44.3	4.3	44.0	38.0	50.0	33.1-55.5°	28.2-38.9	49.4-60.7	Normal
	Adult	22	41.2	3.8	41.0	35.0	47.0	33.1-49.4 ^s	30.9-35.4	46.9-51.8	Normal
TPP (g/L)	Total	30	41.9	7.0	40.0	28.0	60.0	27.3-56.4°	24.0-30.9	52.6-60.0	Normal
	Juvenile	8	42.3	4.9	43.0	34.0	50.0	29.8-54.7°	24.7-35.8	48.4-60.3	Normal
	Adult	22	41.7	7.7	40.0	28.0	60.0	25.4-58.0°	21.0-30.1	53.1-62.8	Normal
WBC	Total	29	6.4	1.4	6.5	3.9	9.9	3.4-9.3°	2.7-4.2	8.5-10.1	Normal
(x10 ⁹ /L)	Juvenile	7	7.5	1.5	7.2	5.1	9.9	3.6-11.4°	1.8-5.6	9.3-13.2	Normal
	Adult	22	6.0	1.2	5.9	3.9	8.9	3.4-8.6°	2.7-4.2	7.8-9.4	Normal
Heterophiles	Total	29	5.3	1.2	5.3	3.2	7.8	2.9-7.8 [°]	2.3-3.5	7.1-8.4	Normal
(x10 ⁹ /L)	Juvenile	7	6.2	1.2	5.9	4.6	7.8	3.1-9.3 °	1.7-4.7	7.6-10.8	Normal
	Adult	22	5.0	1.1	5.1	3.2	7.6	2.8-7.3 ^s	2.2-3.4	6.6-8.0	Normal
Lymphocytes	Total	29	0.7	0.4	0.7	0.06	1.6	ND-1.4 ^s	ND-0.1	1.2-1.6	Normal
(x10 ⁹ /L)	Juvenile	7	0.8	0.4	0.7	0.3	1.6	ND-1.9 ^s	ND-0.3	1.3-2.4	Normal
	Adult	22	0.6	0.4	0.6	0.06	1.2	0.0-1.6 ^s	ND-0.1	1.2-2.9	Box-cox
Monocytes	Total	29	0.2	0.2	0.1	0.0	0.7	ND-0.7 ^s	ND-0.0	0.6-0.9	Box-cox
(x10 ⁹ /L)	Juvenile	7	0.3	0.2	0.3	0.05	0.7	0.0-1.2 ^s	0.0-1.2	0.6-2.4	Box-cox
	Adult	22	0.2	0.2	0.1	0.0	0.7	ND-1.7 ^s	ND-0.0	0.5-0.9	Box-cox
Eosinophils	Total	29	0.2	0.1	0.1	0.0	0.6	ND-0.4 ^r	ND	0.3-0.5	Normal
(x10 ⁹ /L)	Juvenile	7	0.2	0.1	0.2	0.0	0.4	ND-0.6 ^s	ND-0.0	0.4-0.7	Box-cox
	Adult	22	0.1	0.1	0.1	0.0	0.6	0.0-0.5 °	0.0-0.0	0.4-0.8	Box-cox
Basophils	Total	29	0.0	0.0	0.0	0.0	0.08	0.0-0.1 ^s	0.0-0.0	0.1-0.1	Box-cox
(x10 ⁹ /L)	Juvenile	7	0.0	0.0	0.0	0.0	0.08	ND	ND	ND	Box-cox
	Adult	22	0.0	0.0	0.0	0.0	0.07	0.0-0.1 ^s	0.0-0.0	0.1-0.1	Box-cox

MIN, minimum; MAX, maximum; RI, reference interval; L, lower; REF LIM, reference limits; U, upper; Cl, confidence interval; DIST, distribution; ND, not determined; S, standard, R, robust; WBC, white blood cell count; PCV, packed cell volume; TPP, total plasma protein.

Table 2. Biochemical parameters of rehabilitated juvenile and adult Black-chested Buzzard-Eagles Geranoaetus melanoleucus.

ANALYTE		Ν	MEAN	SD	MEDIAN	MIN	MAX	RI	L REF LIM 90% CI	U REF LIM 90% CI	DIST
Ca (mmol/L)	Total	30	2.7	0.3	2.7	2.1	3.2	2.1-3.2 ^s	2.0-2.3	3.1-3.3	Normal
	Juvenile	8	2.9	0.1	2.9	2.7	3.0	ND-3.1 ^s	ND-2.7	3.0-3.1	Box-cox
	Adult	22	2.6	0.2	2.5	2.1	3.2	2.1-3.1 ^s	1.9-2.2	3.0-3.3	Normal
P (mmol/L)	Total	29	1.6	0.7	1.4	0.7	3.2	0.6-3.7 ^s	0.5-0.8	2.9-4.6	Box-cox
	Juvenile	8	2.1	0.9	1.9	1.1	3.2	0.6-5.9°	0.4-1.1	3.3-10.6	Box-cox
	Adulto	21	1.4	0.6	1.3	0.7	2.6	0.4-3.0 ^s	0.3-0.6	2.4-3.5	Box-cox
UA	Total	25	307.4	133.3	297.4	124.9	594.8	100.1-647.4 ^s	81.2-135.8	530.4-781.4	Box-cox
(µmol/L)	Juvenile	6	271.6	77.2	294.4	124.9	333.1	57.4-485.8°	ND- 176.4	361.5- 598.7	Normal
	Adult	19	318.7	146.6	297.4	124.9	594.8	55.2-681.7 [°]	ND-126.5	544.0- 842.7	Box-cox
СК	Total	28	1113.1	404.7	1064.5	515	1995	268.0-1958.1°	66.4-483.8	1731.2-2175.4	Normal
(U/L)	Juvenile	7	1029.1	432.3	1076.0	633	1868	ND-2160.1 ^s	ND-479.8	1551.4-2690.7	Normal
	Adulto	21	1141.0	402.2	1053.0	515	1995	282.4-1999.7°	49.2-536.1	1733.1-2254.7	Normal
CRE	Total	30	42.2	13.2	42.1	21.6	78.4	18.4-69.6°	8.5-21.6	62.5-76.3	Normal
(µmol/L)	Juvenile	8	39.2	13.7	36.4	21.6	56.7	13.2-84.4 ^s	6.9-23.2	57.7-118.8	Box-cox
	Adult	22	43.3	13.1	42.1	27.5	78.4	15.4-71.2 °	7.9-23.4	62.7-79.3	Normal
LDH	Total	27	1393.1	482.0	1172.0	825.3	2321	701.1-2760.6 ^r	653.9-774.3	2055.0-3263.2	Box-cox
(U/L)	Juvenile	8	1489.4	589.1	1208.5	825.3	2321	11.9-2966.9°	ND-720.3	2222.7-3637.2	Normal
	Adult	19	1352.5	441.3	1172.0	851.0	2321	684.5-2715.8°	577.0-825.4	2058.3-3515.7	Box-cox
GGT	Total	30	5.5	1.4	5.5	2.6	8	2.6-8.4 ^s	1.9-3.3	7.6-9.1	Normal
(U/L)	Juvenile	8	6.1	1.5	6.4	3.9	8	2.2-9.9°	0.6-4.1	8.0-11.7	Normal
	Adult	22	5.3	1.3	5.4	2.6	7.5	2.5-8.0 ^s	1.8-3.3	7.2-8.8	Normal
ALP	Total	29	49	33.4	36	18	151	16.1-160.2°	14.3-19.3	104.7-239.1	Box-cox
(U/L)	Juvenile	7	40.9	29.7	24	18	101	ND-181.3 ^s	ND-17.8	75.0-337.3	Box-cox
	Adult	22	51.6	34.7	39	22	151	17.7-151.1 [°]	16.5-20.8	103.6-209.0	Box-cox

MIN, minimum; MAX, maximum; RI, reference interval; L, lower; REF LIM, reference limits; U, upper; CI, confidence interval; DIST, distribution; ND, not determined; S, standard, R: robust; Ca, calcium; P, inorganic phosphorus; UA, uric acid; CK, creatine kinase, CRE, creatinine; LDH, lactate dehydrogenase; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase.

Table 3. Hematological parameters of rehabilitated Black-chested Buzzard-Eagle Geranoaetus melanoleucus, comparing sex.

ANALYTE		Ν	MEAN	SD	MEDIAN	MIN	MAX	RI	L REF LIM 90% CI	U REF LIM 90% CI	DIST
PCV %	Total	29	42.0	4.1	42.0	35.0	50.0	33.4-50.5 °	31.4-35.6	48.2-52.6	Normal
	Male	8	42.6	2.9	43.0	38.0	46.0	35.4-49.8°	32.4-38.9	46.2-53.1	Normal
	Female	21	41.7	4.5	41.0	35.0	50.0	32.1-52.3 °	29.5-34.9	48.3-54.2	Normal
TPP (g/L)	Total	29	41.2	6.2	40.0	28.0	54.0	28.3-54.2°	25.3-31.6	50.7-57.4	Normal
	Male	8	41.5	6.7	39.0	34.0	54.0	24.6-58.4 ^s	17.6-32.7	49.9-66.1	Normal
	Female	21	41.1	6.2	40.0	28.0	50.0	28.0-54.3 °	24.4-31.9	50.2-58.2	Normal
WBC	Total	29	6.4	1.4	6.5	3.9	9.9	3.4-9.3 ^s	2.7-4.2	8.5-10.1	Normal
(x10 ⁹ /L)	Male	8	6.1	1.0	5.8	4.8	7.9	3.7-8.5 ^s	2.8-4.9	7.3-9.6	Normal
	Female	21	6.5	1.6	6.8	3.9	9.9	3.1-9.8°	2.2-4.1	8.8-10.8	Normal
Heterophiles	Total	29	5.3	1.2	5.3	3.2	7.8	2.9-7.8 ^s	2.3-3.5	7.1-8.4	Normal
(x10 ⁹ /L)	Male	8	5.3	0.9	5.3	4.2	6.4	3.2-7.5 [°]	2.3-4.2	6.4-8.5	Normal
	Female	21	5.3	1.3	5.2	3.2	7.8	2.5-8.1 ^s	1.8-3.4	7.2-8.9	Normal
Lymphocytes	Total	29	0.7	0.4	0.7	0.06	1.6	ND-1.4 ^s	ND-0.1	1.2-1.6	Normal
(x10 ⁹ /L)	Male	8	0.5	0.4	0.5	0.06	1.2	0.0-2.3 °	ND-0.1	1.2-4.0	Box-cox
	Female	21	0.7	0.4	0.7	0.12	1.6	0.1-1.6 ^s	0.0-0.2	1.3-2.0	Box-cox
Monocytes	Total	29	0.2	0.2	0.1	0.0	0.7	ND-0.7 ^s	ND-0.0	0.6-0.9	Box-cox
(x10 ⁹ /L)	Male	8	0.1	0.2	0.1	0.0	0.4	0.0-1.4 ^s	0.0-0.0	0.4-4.6	Box-co
	Female	21	0.3	0.2	0.3	0.0	0.7	0.0-0.8 ^s	ND-0.0	0.6-1.0	Box-co
Eosinophils	Total	29	0.2	0.1	0.1	0.0	0.6	ND-0.4 ^r	ND-ND	0.3-0.5	Normal
(x10 ⁹ /L)	Male	8	0.1	0.1	0.1	0.0	0.14	ND-0.2 ^s	ND-0.0	0.1-0.3	Norma
	Female	21	0.2	0.1	0.1	0.0	0.6	ND-0,5 ^r	ND-0.0	0.4-0.6	Norma
Basophils	Total	29	0.0	0.0	0.0	0.0	0.08	0.0-0.1 ^s	0.0-0.0	0.1-0.1	Box-co
(x10 ⁹ /L)	Male	8	0.0	0.0	0.1	0.0	0.07	0.0-0.1 ^r	ND	ND	Normal
	Female	21	0.0	0.0	0.0	0.0	0.08	0.0-0.1 ^s	0.0-0.0	0.0-0.1	Box-co

MIN, minimum; MAX, maximum; RI, reference interval; L, lower; REF LIM, reference limits; U, upper; CI, confidence interval; DIST., distribution; ND, not determined; S, standard, R, robust; WBC, white blood cell count; PCV, packed cell volume; TPP, total plasma proteins.

Table 4. Biochemical parameters of rehabilitated Black-chested Buzzard-Eagle Geranoaetus melanoleucus, comparing sex.

ANALYTE		N	MEAN	SD	MEDIAN	MIN	MAX	RI	L REF LIM 90% CI	U REF LIM 90% CI	DIST
Са	Total	30	2.7	0.3	2.7	2.1	3.2	2.1-3.2 ^s	2.0-2.3	3.1-3.3	Normal
(mmol/L)	Male	8	2.6	0.2	2.5	2.4	3.0	2.3-ND ^r	2.0-2.4	2.9-ND	Normal
	Female	22	2.7	0.3	2.7	2.1	3.2	2.1-3.3°	2.0-2.3	3.1-3.4	Normal
Р	Total	29	1.6	0.7	1.4	0.7	3.2	0.6-4.7 ^s	0.5-0.8	2.9-4.6	Box-cox
(mmol/L)	Male	8	1.5	0.5	1.4	0.9	2.5	0.7-3.6°	0.6-0.9	2.2-5.8	Box-cox
	Female	21	1.7	0.8	1.4	0.7	3.2	0.6-4.6 ^s	0.5-0.8	3.4-7.0	Box-cox
UA	Total	27	350.3	200.8	315.2	124.9	915.9	107.2-920.1 ^s	88.9-137.3	674.8-1188.9	Box-cox
(µmol/L)	Male	7	337.3	183.3	297.4	142.8	594.8	ND-816.7 ^s	ND- 104.5	558.7- 1041.7	Normal
	Female	20	354.8	2111.0	318.2	124.9	915.9	113.3-961.2 ^s	99.9-139.1	682.6- 1267.4	Box-cox
СК	Total	28	1113.1	404.7	1064.5	515	1995	268.0-1958.1°	66.4-483.8	1731.2-2175.4	Normal
(U/L)	Male	7	1393.7	371.0	1367.7	764	1940	423.1-2364.3°	ND-922.3	1842-2819.7	Normal
	Female	21	1019.5	378.2	986.0	515	1995	495.8-2106.8 ^s	423.3-594.0	1630.2-2767.6	Box-cox
CRE	Total	30	42.2	13.2	42.1	21.6	78.4	18.4-69.6°	8.5-21.6	62.5-76.3	Normal
(µmol/L)	Male	8	48.1	15.6	45.6	27.5	78.4	20.3-102.4°	13.3-30.7	68.7-151.8	Box-cox
	Female	22	40.0	11.8	36.1	21.6	68.9	14.9-65.2°	8.2-22.1	57.5-72.5	Normal
LDH	Total	27	1393.1	482.0	1172.0	825.3	2321	701.1-2760.6 ^r	653.9-774.3	2055.0-3263.2	Box-cox
(U/L)	Male	6	1303.9	444.1	1108.5	918.3	1979	70.8-2536.9°	ND-756.0	1821.0-3186.5	Normal
	Female	21	1418.5	499.6	1172.0	825.3	2321	714.7-2896.3°	633.9-837.3	2215.5-3579.2	Box-cox
GGT	Total	30	5.5	1.4	5.5	2.6	8	2.6-8.4 ^s	1.9-3.3	7.6-9.1	Normal
(U/L)	Male	8	4.9	1.5	4.8	2.6	6.8	1.2-8.5 [°]	ND-3.0	6.7-10.2	Normal
	Female	22	5.7	1.3	5.9	2.8	8	2.9-8.5°	2.1-3.7	7.7-9.3	Normal
ALP	Total	29	49	33.4	36	18	151	16.1-160.2 ^s	14.3-19.3	104.7-239.1	Box-cox
(U/L)	Male	8	58.1	28.8	26	108	42.03	19.3-249.4 ^s	15.1-28.5	102.7-703.2	Box-cox
	Female	21	45.6	33	35	18	151	15.6-152.9°	14.3-18.3	90.6-232.3	Box-cox

MIN, minimum; MAX, maximum; RI, reference interval; L, lower; REF LIM, reference limits; U, upper; CI, confidence interval; DIST, distribution; ND, not determined; S, standard, R: robust; Ca, calcium; P, inorganic phosphorus; UA, uric acid; CK, creatine kinase, CRE, creatinine; LDH, lactate dehydrogenase; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase.

juveniles (P < 0.05) were found in Ca, P, WBC, and heterophils values, with higher values in juveniles. Significant differences were also found in CK and eosinophil values between males and females (P < 0.05) (Tables 3 and 4). We did not find blood parasites in blood smears.

The mean PCV and TPP values were similar to those obtained by Black et al. 2011 in Bald Eagles *Haliaetus leucocephalus*. Our study reported a wide range in WBCs, being heterophils the most numerous leukocytes, followed by lymphocytes, monocytes, eosinophils, and basophils, as also found in Bald Eagles (Mealey et al. 2004). These results for WBC were lower than those reported in Spanish Imperial Eagles *Aquila adalberti* (García-Montijano et al. 2002), Golden Eagles *Aquila chrysaetos* (Nazifi et al. 2008), and Harpy Eagle *Harpia harpyja* (Oliveira et al. 2014).

The serum totals for Ca and P concentration were similar to those of other adults Accipitridae, such as Spanish Imperial Eagles and Golden Eagles (García-Montijano et al. 2002, Nazifi et al. 2008). Values of LDH and CRE were similar to those obtained in Golden Eagles (Nazifi et al. 2008). While the mean values determined for CK were higher than those of the Golden Eagle, the Booted Eagle *Hieraaetus pennatus*, and the Spanish Imperial Eagle (Casado et al. 2002, García-Montijano et al. 2002, Nazifi et al. 2008). The results obtained for ALP were within the range of normal activity, but with a very wide range of variation compared to Booted Eagles and Golden Eagles (Casado et al. 2002, Nazifi et al. 2008).

Samples of blood collected from these captive Black-chested Buzzard-Eagles, before release, can be used as normal hematologic parameters and serum chemistry; however, future research should be directed toward samples from wild populations.

ACKNOWLEDGMENTS

The authors would like to thank Centro de Rehabilitación de aves rapaces de la Unión de Ornitólogos de Chile (and personally Jürgen Rottmann and Francisca Izquierdo); and to project Fondecyt 1130948 and The National Agency for Research and Development (ANID) DOCTORADO BECAS CHILE/2018 – 72190138. The authors would like to express their deepest gratitude to Daniel González-Acuña who sadly passed away before this paper was published.

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