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Authors: del Pilar Lanzarot, Maria, Montesinos, Andrés, San Andrés, Manuel Ignacio, Rodríguez, Casilda, and Barahona, María Victoria

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HEMATOLOGICAL, PROTEIN ELECTROPHORESIS AND CHOLINESTERASE VALUES OF FREE-LIVING NESTLING PEREGRINE FALCONS IN SPAIN

Maria del Pilar Lanzarot,[1,3] Andrés Montesinos,[2] Manuel Ignacio San Andrés,[1] Casilda Rodríguez,[1] and María Victoria Barahona[1]

[1] Departamento de Toxicología y Farmacología, Facultad de Veterinaria, Universidad Complutense de Madrid, Avda./Puerta de Hierro s/n, 28040, Madrid, Spain
[2] Centro Veterinario Los Sauces, C/ Murillo n° 3, 28015, Madrid, Spain
[3] Corresponding author (e-mail: mplanzarot@mx4.redestb.es)

ABSTRACT: Protein electrophoresis, hematological and cholinesterase values were determined in 32 nestling free-living peregrine falcons (*Falco peregrinus*) (15- to 27-days-old) in order to establish normal reference values for this population. The following values (mean ± SD) were observed: prealbumin 0.31 ± 0.04 g/dl, albumin 1.25 ± 0.06 g/dl, α_1 and α_2-globulin 0.23 ± 0.02 and 0.16 ± 0.02 g/dl respectively, β-globulin 1.02 ± 0.05 g/dl, γ-globulin 0.060 ± 0.08 g/dl, total protein 3.79 ± 0.18 g/dl, 21.26 ± 1.30 white blood cells/μl (1×10^3), 2.17 ± 0.07 red blood cells/μl (1×10^6), packed cell volume 37.58 ± 0.82%, hemoglobin 20.96 ± 0.29 g/dl, heterophils 61.14 ± 2.50% and cholinesterase 1,184 ± 75 IU/L. There were no difference in any of these parameters among males and females. The hematological values obtained could be considered as representative values in free-living nestling peregrine falcons.

Key words: Cholinesterase, electrophoresis, *Falco peregrinus*, hematology, nestling peregrine falcons.

INTRODUCTION

The peregrine falcon (*Falco peregrinus*) is a polytypic species of cosmopolitan distribution (Orta et al., 1994). In Spain, the peregrine falcon is protected by the local Law 4/89 of the Conservation of Nature and Flora and Wild Fauna (B.O.E., 27 March 1989) and is CITES I.

It is well known that clinical hematological and chemistry data can be useful aids for diagnosis of disease in birds (Karesh et al., 1997; Toro et al., 1997; Villouta et al., 1997). However, diagnostic interpretation of blood data requires knowledge of the normal hematological values for each species, since there can be large differences among some avian species. Moreover, it is important to take into consideration additional factors such as age and sex as these tend to determine the blood profile of an avian species (Clubb et al., 1991b, c; D'Aloia et al., 1996; McInnes et al., 1996; Villouta et al., 1997).

Reference values for different plasma chemical variables in adult falcons have been recently established (Redig, 1993; Cray and Tatum, 1998; Lumeij et al., 1998). However, there is very little information available on chemistry and hematological values for free-living nestling peregrine falcons. Therefore, the aim of this investigation was to obtain a representative hematological range for free-living nestling of this species and evaluate whether or not these parameters varied with age and sex.

MATERIALS AND METHODS

In conjunction with an ongoing genetic identification and banding, evaluations of individual peregrine falcon and sample collections for health analysis were conducted during May and June 1998, the hatching-fledging period for this species in this area. The study was conducted on 32 free-living nestling peregrine falcons (48% males and 58% females) caught in central Spain (3°35′W, 40°25′N).

The nestlings were (caught by hand from the nest) tacked from the nest ledge, lowered down to the ground or brought up the top of the cliff in a backpack containing different plastic bags for each nest, for study, and then returned. Each bird was handled by the same veterinarian for approximately 10 min for weighing, physical examination, and the blood sample collection (n = 32) was performed. Ages of all birds were known with an accuracy of 2 to 3 days. Chicks ranged in age from 15- to 27-days-old. The determination of the sex is based in

the weight in conjunction with the development. Blood samples were collected from either ulnaris or jugular veins using 2 ml disposable syringes and 25 gauge needles.

After collection, the samples were mixed immediately with the anticoagulant dipotassium EDTA (1.5 mg/dl) and maintained at refrigerated temperatures (4–6 C). Processing of blood samples occurred in the laboratory within 12 hr.

For the white blood cell counts (WBC) the whole blood was diluted 50 times using Natt and Herrick's solution (Natt and Herrick, 1952) in blood-cell dilution pipettes. Red blood cell counts (RBC) were made using the method Unopette (Unopette Test 5877, Becton-Dickinson Vacutainer Systems, Rutherford, New Jersey, USA). Using an improved Neubauer hemocytometer (Brand, Wertheim, Germany), the red blood cells seen in the 10 groups of 16 small squares and all the white blood cells seen in the Neubauer slide were counted (Campbell, 1995). The hemoglobin (Hb) content was determined using the Drabkin technique modified by addition of distillated water as the hemolytic agent (Drabkin, 1945). Packed cell volumes (PCV) were determined by centrifugation at 3,000 $\times$ *g* for 5 min. Mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the following equations (Campbell, 1995): MCV = (PCV/RBC)·10; MCH = (Hb/RBC)·10; and MCHC = (Hb/PVC)·100.

Protein electrophoresis was developed using cellulose acetate (Lumeij, 1987). Total protein (TP) was determined with the Biuret method (Lumeij and MacLean, 1996). Thin blood smears were stained with a Diff-Quick stain (Panreac, Barcelona, Spain) and at least 400 white blood cells were counted in each smear. Blood parasites were detected by screening 10,000 red blood cells with a 100$\times$ objective of a light microscope. In agreement with Phalen et al. (1995), if less than one parasite per 100 white blood cells was found, the bird was considered infected; it was discarded for this study and the concentration of the organisms was not calculated. Butyrilcholinesterase (ChE, E.C.3.1.1.8) was determined in plasma with a test kit whereby butyrilcholine was used as a substrate (Test Combination 124125, Boehringer Mannheim, Mannheim, Germany).

Values are expressed by arithmetic mean, standard deviation (SD) and inner limits of the percentiles P_5 and P_{95} (for 15 plasma variables). Normality was assesed with the Kolmogorov-Smirnov statistic with Lilliefors significance correction, the skewness statistic and visual examination of data distribution. Comparisons were performed either by Mann-Whitney *U*-test (non-parametric analysis) or by one way analysis of variance (parametric analysis) (Bolton, 1997). Differences were considered significant at $P < 0.05$. Statistical calculations were performed using SPSS 6.1 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

The mean body weight ($\pm$SD) was 562.57 $\pm$ 28.57 g for all birds. Females weighed 691.15 $\pm$ 40.41 g and males had a weight of 530.00 $\pm$ 30.89 g. All but four birds appeared to be in good condition, with no abnormality noted during physical examination, hematological analysis throughout the capturing period. Four birds were excluded from the descriptive statistics: two of them showed detectable abnormalities such as emaciation and a dislocated leg and the other two had hemoparasites (*Plasmodium* spp. and *Haemoproteus* spp.). Representative blood smears were deposited in the Ciencias Naturales Museum (Madrid, Spain; accession numbers 7013175 and 7013166).

Hematological and blood chemistry values are shown in Tables 1 and 2. We observed that butyrilcholinesterase, β-globuline, total proteins, heterophils, lymphocytes, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell counts and red blood cell counts did not have a Gaussian distribution. No difference in any parameter examined was found between males and females ($P > 0.05$), only the female weights were significantly higher than the male weights ($P < 0.05$).

DISCUSSION

This study shows hematological values, protein electrophoresis and cholinesterase values from a sufficiently large population of free-living nestling peregrine falcons to consider these values as statistically valid. Previously, Lumeij et al. (1998) indicated that these kinds of data are not fitted to a normal distribution. Similarly we found that some parameters such as ChE, β-globuline, total proteins, heterophils, lym-

TABLE 1. Blood chemistry values for nestling free-living peregrine falcons in Spain.

Parameter		Mean ± SD	Median	P_5–P_{95}
Prealbumin	(g/dl)	0.31 ± 0.04	2.00	0.10–0.87
	(%)	8.35 ± 1.15	5.97	2.73–24.39
Albumin	(g/dl)	1.25 ± 0.06	1.20	0.74–1.91
	(%)	34.30 ± 1.31	34.60	23.35–44.73
α_1-globulin	(g/dl)	0.23 ± 0.02	0.20	0.10–0.53
	(%)	6.43 ± 0.66	5.03	2.28–12.81
α_2-globulin	(g/dl)	0.16 ± 0.02	0.15	0.06–0.53
	(%)	4.08 ± 0.36	3.63	1.93–9.62
β-globulin	(g/dl)	1.02 ± 0.05	1.05	0.49–1.50
	(%)	28.11 ± 0.84	28.00	17.70–36.10
γ-globulin	(g/dl)	0.60 ± 0.08	0.40	0.24–1.86
	(%)	16.28 ± 1.20	14.50	9.45–34.10
Total Protein	(g/dl)	3.79 ± 0.18	3.80	2.54–5.96
A/G Ratio		0.80 ± 0.03	0.79	0.46–1.17
ChE	(IU/l)	1184.00 ± 74.99	1210.50	509.45–1802.05

phocytes, MCH, MCHC, WBC and RBC do not have normal distribution.

The mean WBC for the free-living nestling peregrine falcons is higher than the values reported for adults (Redig, 1993; Jennings 1996). The age-related variation of WBC is similar to that reported for Psittacines (Clubb et al., 1991a; Karest et al., 1997) and for white storks (*Ciconia ciconia*) (Montesinos et al., 1997). It has been suggested that exposure to antigens in the wild setting or more responsive immune systems in the young birds are both possible explanations for their higher mean WBC values (Karesh et al., 1997). Heterophils (61.14 ± 2.5%) are the most numerous leukocytes of the blood of nestlings as reported previously for adult peregrine falcons (Redig, 1993).

Commonly encountered stressors include travel, handling, restraint, sample collection technique and concomitant disease. In general, the absolute heterophils and lymphocyte counts are susceptible to variations under the influence of stress (Breazile 1988; Gross and Siegel, 1983). We have not found any meaningful differences in individual hematological parameters attributable to stress-influenced hemograms of the peregrine falcon nestlings.

The RBC and PCV for the chicks are lower than values reported for adult peregrine falcons by Jennings (1996) and Rosskopf and Woerpel (1996), which suggest

TABLE 2. Hematological findings in nestling free-living peregrine falcons in Spain.

Parameter	Mean ± SD	Median	P_5–P_{95}
WBC/μl ($\cdot 10^3$)	21.26 ± 1.30	20.6	11.55–39.23
RBC/μL ($\cdot 10^6$)	2.17 ± 0.07	2.04	1.27–2.86
PCV (%)	37.58 ± 0.82	38.55	31.80–44.84
Hb (g/dl)	20.96 ± 0.29	21.30	17.54–23.00
MCV (fl)	179.37 ± 8.24	165.69	123.90–304.83
MCHC (g/dl)	56.56 ± 1.54	55.70	42.53–68.69
MCH (pg)	100.16 ± 4.43	99.25	72.01–160.56
Heterophils (%)	61.14 ± 2.50	61.00	38.80–82.65
Lymphocytes (%)	34.89 ± 2.54	36.00	12.90–58.65
Monocytes (%)	1.53 ± 0.26	1	0.00–4.55
Eosinophils (%)	2.18 ± 0.31	2.00	0.00–5.55
Basophils (%)	0.07 ± 0.04	0	0.00–1.00

an age influence in these parameters. The occurrence of lower Hb and RBC levels in young than adult birds has been reported in red-tailed hawk (*Buteo jamaicensys*) and Rosy and Chilean flamingos (*Phoenicopterus ruber ruber* and *P. ruber chiliensis*, respectively) (Hawkey et al., 1984a, b), white stork (*Ciconia ciconia*) (Montesinos et al., 1997), domestic fowl, geese and quail (Hodges, 1977), and may be characteristic of all avian species (Hawkey et al., 1984b). Montesinos et al., (1997) indicated that these variations were probably due to their adaptation to flight, at which time the need for oxygen was greatly increased.

We found that the mean value for hemoglobin (20.96 ± 0.29 g/dl) obtained in this study is very close to that reported for adult *Falco peregrinus* (11.6–19.1 g/dl) and *Accipiter gentilis* (16.0–21.6 g/dl) (Heidenreich, 1997). Moreover, Rosskopf and Woerpel (1996) show a value of 23.5 g/dl for peregrine falcon, based on citrated blood, as typical hemoglobin data in automated hemogram.

The range for total protein is in accordance with those obtained for adult peregrine falcons (Lumeij et al., 1998) and other birds of prey such as captive and free living condors (*Vultur gryphus*) (Toro et al., 1997) and Mauritius kestrel (*Falco punctatus*) (Cooper et al., 1986).

Protein electrophoresis is a valuable diagnostic aid when abnormal total protein values are found. On the other hand, Cray and Tatum (1998) indicated that results of protein electrophoresis are often abnormal when the total protein concentration is within reference ranges. However, the first step in the application of protein electrophoresis in the diagnosis of raptor diseases is the determination of the normal values and physiologic variations of the electrophoresis pattern. In general, concentrations of α_1-, α_2- and β-globulins tend to be significantly higher in raptors than in other avian species with maximum reference concentrations reaching 27%, 16% and 30%, respectively, of the total protein concentration whereas, the γ-globulin is within the reference range typical of other birds (Cray and Tatum, 1998). Accordingly, the concentration of the six protein fractions resolved by protein electrophoresis (prealbumin, albumin, α_1-, α_2- β-, and γ-globulins) in nestling falcons are similar to those described for other raptors (Toro et al., 1997; Cray and Tatum, 1998).

Prealbumin constituted 19.5% of the total albumin in nestlings. Prealbumin concentrations can vary markedly between avian species, it can be represent from 10 to 75% of the total albumin concentration (prealbumin + albumin) or be absent entirely (Cray and Tatum, 1998).

Birds, especially young or small birds, are much more susceptible to anticholinesterase insecticide. There are a large number of organophosphorus (OP) and carbamate compounds and most inhibit cholinesterase enzymes (ChE) (Fossi et al., 1994). Direct measurement of OP and carbamate insecticide residues from operational applications is difficult and often impractical because these insecticides are quite labile in biological tissues and a large number of compounds are in use. Although pseudocholinesterase activities have predictive value, erythrocyte cholinesterase activity is more often used to predict and monitor activity of neural acetylcholinesterase (AChE) in mammals. This enzyme is absent in avians (Farage-Elawar et al., 1988). However, measurement of ChE activity or ChE inhibition in brain tissue or blood plasma of birds can be used as an indicator of exposure to ChE-inhibiting insecticides (McInnes et al., 1996) and therefore we used a butyrylcholinesterase (BChE) activity to know the levels of this enzyme in nestling free-living falcons. The results obtained in this work are higher than those described in adult birds by Lumeij et al., (1998), although the methodology used was similar. In birds, the proportion of plasma ChE that is BChE or AChE varies for different species and different species have different age-dependent patterns of AChE or BChE ac-

tivity (McInnes et al., 1996). In eastern bluebirds (*Sialia sialia*) and European starlings (*Sturnus vulgaris*) (Gard and Hooper, 1993), plasma BChE increased over the nestling period. In a comparable study, Grue at al. (1981) observed an increase in plasma BChE activity in nestling bluebirds and starlings. It could be due to a decrease in the cholinesterase level with the age.

Allen (1988) indicated that different factors such as age and sex may critically affect the blood chemistry values. In the present work, we observed no differences among the measured parameters between females and males ($P > 0.05$), which is in agreement with Toro et al. (1997). By contrast, sex-related differences in condors for aspartate amino transferase and calcium have been reported by Gee et al. (1981).

In conclusion, the hematological and clinical chemistry results of free-living peregrine falcons obtained in this study can be considered as representative values for chick birds of this species. We found that many of the hematological parameters differed significantly in accordance with age.

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