HEMATOLOGICAL AND BIOCHEMICAL ANALYSES OF MAGNIFICENT FRIGATEBIRD *FREGATA MAGNIFICENS* FOUND ON THE COAST OF THE STATE OF RIO DE JANEIRO, BRAZIL

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ABSTRACT

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The Magnificent Frigatebird *Fregata magnificens* is prevalent along the Brazilian coast and is classified as Least Concern by the IUCN. Hematological and biochemical analyses can be used to assist in the diagnosis of diseases and to monitor the population of this species and others, but the scarcity of species-specific reference parameters hinders the interpretation of acquired information. To help alleviate this situation, we describe reference values for hemograms and the biochemistry of Magnificent Frigatebirds found on the coast of the State of Rio de Janeiro, Brazil, 2017–2018.

Key words: Brazil, Magnificent Frigatebird, hematology, parameters, *Fregata magnificens*, rehabilitation

INTRODUCTION

The Magnificent Frigatebird *Fregata magnificens* is a tropical seabird that is common along the Brazilian coast; the species is widely distributed in the Atlantic and in the south and central Pacific (Sick 1997, Schreiber & Burger 2001). The species is large, having a two-meter wingspan but weighing only 1.5 kg; such characteristics lead to its soaring flight style (Weimerskirch *et al.* 2003). Magnificent Frigatebirds are long-lived, can reach 30 years of age, and feed by kleptoparasitism (Diamond 1973, Schreiber & Burger 2001). According to the Red List of Threatened Animals, it is categorized as Least Concern because its population is increasing (BirdLife International 2018).

Like many seabirds, little is known of frigatebird physiology. Hematological and biochemical tests, however, can assist in the diagnosis of diseases, thus improving the clinical evaluation of individuals brought to wildlife care facilities. Such testing is becoming increasingly important in the monitoring of wild animal populations (Alonso-Alvarez 2005, Lewbart *et al.* 2014), but it remains scarce in marine animal medicine (Alonso-Alvarez 2005).

Hematological and biochemical parameters for *F. magnificens* are scarce in the literature, which increases the risk of inaccurate diagnoses for individuals brought to wildlife care facilities (Work 1996). In that regard, the present study describes reference values for hemograms and blood chemistry in Magnificent Frigatebirds found on the coast of the State of Rio de Janeiro, Brazil.

METHODS

The individual birds in the present study were received by rehabilitation centers between January 2017 and October 2018. Admitted individuals were either clinically healthy but admitted for tenorrhaphy surgery to correct a wing injury, or they showed signs of exhaustion. Under physical restraint, venipuncture of the ulnar vein was done using a 3-mL syringe and a 13 × 4.5 mm needle. Blood was collected in two pediatric tubes—one contained anticoagulant (heparin) and one was a clot-activating tube. A smear was made with blood from the heparinized sample and fixed with Diff-Quick (RenyLab®), and blood from the coagulated tube was used to measure glucose (Accu-chek Active® glucometer) according to the manufacturer's instructions. To obtain hematocrit values, capillary tubes were read after centrifugation at 12 000 rpm. Subsequently, total protein plasma concentrations were obtained using a portable refractometer (RTP-20ATC). Erythrocyte and leukocyte counts were performed in a Neubauer chamber using a 400× magnification. We diluted 10 μL of heparinized whole blood with 1 mL of 0.9% aqueous NaCl solution in a test tube with a five-minute interval for sedimentation, then pipetted 10 μL to fill a Neubauer chamber receptacle. The counts were performed using 400× magnification of five central squares for erythrocytes and four lateral squares for leukocytes. The hematological indices were obtained by simple mathematical calculation. The blood smears were stained with Diff-Quick as per the manufacturer's instructions (RenyLab®). Biochemical analysis was conducted in a Bioplus® automated biochemical apparatus (Bio 200 model) using commercial Labtest® kits for uric acid, creatinine, alkaline phosphatase, alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

Weights were measured using a digital platform scale. Physical measurements were taken using a tape measure. Sexing was determined by plumage characteristics, which are apparent among adults; juveniles do not display sexual dimorphism (Diamond 1973, Schreiber & Schreiber 1988, Sick 1997) and could not be sexed.

Results are presented as the mean, standard deviation, and 95% confidence intervals. *P* values of ≤ 0.05 were considered statistically significant. As the data did not follow a normal distribution even after the transformation, the concentrations in the groups (males and females, juveniles and adults) were compared using a non-parametric Mann-Whitney test. The analyses were performed in the statistical application R (R Development Core Team 2017), using a level of 5% significance.

RESULTS AND DISCUSSION

In total, 66 frigatebirds were analyzed: 36 (54.0%) adult females, 22 (33.5%) adult males and 8 (12.5%) juveniles. Adult females weighed 1.00–1.80 kg (1.45 ± 0.16 kg), adult males weighed 1.00–1.70 kg (1.18 ± 0.16 kg) and juveniles weighed 1.00–1.65 kg (1.23 ± 0.22 kg). Adult males were 85.0–101.3 cm in length, adult females were 87.0–105.0 cm, and juveniles were 80.0–100.0 cm, with a mean of 94.7 ± 5.2 cm for all three groups.

Samples from 28 individuals were used for the biochemical analysis (Tables 1, 2): 9 (32.14%) adult females, 10 (35.72%) adult males,

TABLE 1
Results of hematological analysis, according to age, for Magnificent Frigatebirds found along the coast of Rio de Janeiro, Brazil, 2017–2018

Analyses[a]	Juveniles (*n* = 8)	Adults (*n* = 58)	M-W	*P*	Females (*n* = 36)	Males (*n* = 22)	M-W	*P*
Erythrogram								
Erythrocytes (10^6/µL)	2.77 ± 0.42 (1.94–3.59)	3.01 ± 0.22 (2.58–3.44)	1955.0	0.8212	3.24 ± 0.30 (2.65–3.83)	2.63 ± 0.28 (2.07–3.19)	1144.0	0.1915
Hemoglobin (g/dL)	14.58 ± 0.93 (12–16)	14.08 ± 0.65 (12–15)	1884.5	0.2532	14.74 ± 0.98 (12–16)	13.00 ± 0.53 (11–14)	1174.5	0.0720
PCV (%)	44.38 ± 2.85 (38–49)	48.53 ± 8.76 (31–65)	1860.0	0.1042	51.25 ± 3.18 (48–54)	38.23 ± 1.82 (34–41)	1178.0	0.1792
MCV (fL)	185.25 ± 29.40 (127–242)	167.02 ± 9.33 (148–185)	1911.0	0.5360	156.53 ± 11.01 (134–178)	184.18 ± 16.45 (151–216)	990.0	0.2518
MCH (pg)	61.75 ± 9.83 (42–81)	55.16 ± 3.16 (48–61)	1909.5	0.5167	52.06 ± 3.70 (44–59)	60.23 ± 5.69 (49–71)	1000.0	0.3242
MCHC (g/dL)	33.00 ± 0.00 (-)	33.00 ± 0.00 (-)	-	-	33.00 ± 0.00 (-)	33.00 ± 0.00 (-)	-	-
Leukogram								
Leukocytes (/mm³)	12.59 ± 2.59 (7.52–17.66)	14.79 ± 0.99 (12.85–16.73)	1982.5	0.4434	15.76 ± 1.21 (13.39–18.13)	13.20 ± 1.68 (9.90–16.49)	1157.0	0.1298
Heterophils (/mm³)	6774.88 ± 1555.76 (3725–9824)	7932.66 ± 756.09 (6450–9414)	1959.5	0.7532	7178.81 ± 723.56 (5760–8596)	9166.23 ± 1595.70 (6038–12293)	1023.5	0.5425
Eosinophils (/mm³)	244.00 ± 171.55 (0–580)	309.07 ± 63.57 (184–433)	1985.0	0.3767	297.72 ± 66.56 (167–428)	327.64 ± 129.65 (73–581)	1075.0	0.8304
Basophils (/mm³)	145 ± 145 (0–429)	386.31 ± 141.41 (109–663)	1980.5	0.3541	265.44 ± 121.24 (27–503)	584.09 ± 316.23 (0–1203)	1012.5	0.3289
Lymphocytes (/mm³)	2869.38 ± 902.29 (1100–4637)	3825.31 ± 499.19 (2846–4803)	1963.5	0.6944	3939.50 ± 680.81 (2605–5273)	3638.46 ± 718.60 (2230–5046)	1043.5	0.7730
Monocytes (/mm³)	1530.25 ± 1121.58 (0–3728)	1603.48 ± 252.33 (1108–2098)	2007.0	0.2118	1589.56 ± 288.44 (1024–2154)	1626.27 ± 478.20 (689–2563)	1100.5	0.5423
Glucose (mg/dL)	246.63 ± 24.36 (198–294)	254.33 ± 6.62 (241–267)	1955.0	0.8212	259.69 ± 8.81 (242–276)	245.55 ± 9.80 (226–264)	1117.0	0.3823
Total Protein (g/dL)	4.35 ± 0.47 (3.4–5.2)	4.33 ± 0.14 (4.0–4.6)	1939.0	0.9450	4.37 ± 0.17 (4.0–4.6)	4.26 ± 0.22 (3.8–4.8)	1090.5	0.6526

[a] M-W = Mann-Whitney test; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration

and nine (32.14%) juveniles. No hemoparasites were found during the examination. There were no statistical differences between the studied variables, and only the mean alkaline phosphatase was significantly ($P = 0.05$) higher in juveniles (75.67 ± 19.90 IU/L) than in adults (38.95 ± 9.16 IU/L). The mean ALT was significantly higher in adult females (224.56 ± 35.61 IU/L, $P < 0.05$) than in adult males.

We compared our values for weight, hematocrit, total protein, glucose, complete leukogram, uric acid, and AST to those described by Padilla & Parker (2008) and Work (1996) in their study of *Fregata minor*; there are no published values for our other parameters, even for other species of the same genus. Our hematocrit values did not indicate variation between individuals (adults or juveniles) when compared with data previously published by Work (1996). In the latter study, juvenile hematocrits (28%–54%) were lower than those of adults (43%–47%). On the other hand, the averages found in our three sample groups corroborates those reported by Padilla & Parker (2008) (55% ± 8%), including the mean value for glucose (212 ± 45 mg/dL) and total protein (3.6 ± 0.5 g/dL). In the study of *F. magnificens* by Novelino *et al.* (2016), the mean for glucose was higher (311 ± 42 mg/dL) than that found in the present study. This difference may be caused by the lower number of individuals tested by Novelino *et al.* ($n = 8$) or by the jugular method they used to collect blood, as glucose levels increase considerably under stress (Hochleithner 1994).

According to Padilla & Parker (2008), *F. minor* had the highest prevalence of hemoparasites (29.2%) among several seabird species. However, we did not find any hemoparasites. We cannot explain this; although *F. minor* and *F. magnificens* are congeneric, there is the possibility of species-specific hemoparasites, but this theory requires further study.

In the leukogram, there is a wide range of reference parameters for birds compared to mammals (Campbell 1994). The reference range we found for total leukocytes in the three groups of frigatebirds was greater than that described by Work (1996), in which juveniles had considerably lower total leukocyte values (1.65–23.40 × 10^3/μL) compared to adults (3.52–17.58 × 10^3/μL for males and 4.73–21.38 × 10^3/μL for females). The mean was also higher than that found by Padilla & Parker (2008), except for lymphocytes that showed little variation (4.0 × 10^3/μL ± 1.2 × 10^3/μL).

In the biochemical analysis, our alkaline phosphatase values showed a significant ($P = 0.05$) variance between juveniles (75.6 ± 19.9 IU/L) and adults of both sexes (males 25.5 ± 2.9 IU/L and females 53.8 ± 18.2 IU/L), with juveniles having higher values. In addition, our uric acid and AST values were higher among juveniles (8.5 ± 3.0 mg/dL to uric acid and 321.1 ± 85.5 IU/L to AST) compared to adults (6.65 ± 0.70 mg/dL to uric acid and 233.0 ± 31.4 IU/L to AST). This decreasing trend was also observed by Casado *et al.* (2002) and Alonso-Alvarez (2005), and it can be explained by improvement in the nutritional state (Singer 2003); normally, juvenile birds exhibit lower AST and uric acid values compared to adults (Hochleithner 1994).

In contrast, our ALT values were higher in adults of both sexes (173.5 ± 22.2 IU/L) compared to juveniles (166.1 ± 65.3 IU/L) including a significant variance ($P < 0.05$); values of this enzyme are known to increase with age (Hochleithner 1994). Regarding creatinine results, there was no variation among the three groups studied (0.39 ± 0.04 mg/dL for adults and 0.40 ± 0.06 mg/dL for juveniles). Although creatinine levels are directly related to muscle tissue, it is an insensitive parameter, and significant differences have not been shown to exist among bird species, regardless of muscle mass, ranging from 0.3 to 0.9 mg/dL for raptors and 0.1 to 0.2 mg/dL for parakeets (Hochleithner 1994).

In summary, we describe the hematological results of 66 individuals and the biochemical results of 28 individuals of *F. magnificens* found on the Brazilian coast. Unfortunately, owing to lack of information, the only possible comparison was with another bird of the same genus (*F. minor*). However, we agree that, because they are different species, there may be discrepancies in these comparisons.

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TABLE 2
Results of biochemical analysis, according to age, for Magnificent Frigatebirds found along the coast of Rio de Janeiro, Brazil, 2017–2018

Analyses[a]	Juveniles ($n = 9$)	Adults ($n = 19$)	M-W	P	Females ($n = 9$)	Males ($n = 10$)	M-W	P
Uric acid (mg/dL)	8.51 ± 3.08 (2.4–14.5)	6.65 ± 0.75 (5.1–8.1)	282.5	0.7489	6.70 ± 1.16 (4.4–8.9)	6.61 ± 1.04 (4.5–8.6)	93.0	0.8381
ALT (IU/L)	166.11 ± 65.34 (38.0–294.1)	173.53 ± 22.22 (129.9–217.0)	302.0	0.2007	224.56 ± 35.61 (154.7–294.3)	127.60 ± 19.15 (90.0–165.1)	116.5	0.0336
AST (IU/L)	321.11 ± 85.52 (153.5–488.7)	233.00 ± 31.40 (171.4–294.5)	269.0	0.7679	279.11 ± 54.39 (172.5–385.7)	191.50 ± 31.24 (130.2–252.7)	106.0	0.2057
Creatinine (mg/dL)	0.40 ± 0.06 (0.27–0.53)	0.39 ± 0.04 (0.31–0.48)	278.5	0.9004	0.37 ± 0.07 (0.24–0.50)	0.42 ± 0.06 (0.31–0.53)	81.5	0.5076
Alkaline phosphatase (IU/L)	75.67 ± 19.90 (36.6–114.6)	38.95 ± 9.16 (21.0–56.9)	235.5	0.0517	53.89 ± 18.29 (18.0–89.7)	25.50 ± 2.94 (19.7–31.2)	97.5	0.5666

[a] M-W = Mann-Whitney test; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

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