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SERUM CHEMISTRY OF FREE-RANGING NAZCA BOOBIES (*SULA GRANTI*)

Terri J. Maness, Ph.D., and David J. Anderson, Ph.D.

Abstract: This study reports body mass and serum chemistry reference values of 121 male and 57 female Nazca boobies (Sulidae: *Sula granti*) from a colony on Isla Española, Galápagos Islands, Ecuador. Circulating aspartate aminotransferase, creatine kinase, lactate dehydrogenase, total protein, immunoglobulin Y, uric acid, blood urea nitrogen, triglycerides, cholesterol, and creatinine were quantified and analyzed by sex. Sex explained little variance in all examined variables except mass; females were heavier than males, as expected for sulids. Uric acid values had a bimodal distribution, likely reflecting differences in recent foraging success. Aspartate aminotransferase and creatine kinase values were similar to those reported in other sulids. Clinical health reference values are critical for practitioners investigating responses of seabirds to oil spills, coastal restoration efforts, or emerging diseases. These data from a species living in a relatively isolated and pristine area provide a valuable baseline for future health evaluations.

Key words: Clinical chemistry, immune function, seabirds, *Sula granti*, sulids.

BRIEF COMMUNICATION

Nazca boobies (Sulidae: *Sula granti*) are long-lived[1] pelagic seabirds of the eastern tropical Pacific Ocean[8] that have a low reproductive rate, raising at most a single offspring per year from a clutch of one or two eggs.[1,3,19] Males and females have similar parental roles, sharing incubation, brooding, and feeding of nestlings.[3] At Punta Cevallos, Isla Española, Galápagos Islands, the birds breed seasonally, with most egg laying between October and January and most fledging completed by June.[16] Colonial breeding permits collection of large sample sizes, and breeding at the Punta Cevallos colony has been monitored each year beginning in 1984.[3,15] As such, much is known about the demography, behavior, and reproduction of Nazca boobies,[3,13–15] but information on their clinical chemistry is scarce.[18] Baseline clinical chemistry can be used to assess the health status of free-ranging and captive animals.[9] This study reports reference values of free-ranging Nazca boobies from the Punta Cevallos colony and examines the relationships between these values and sex. Seabirds include some of the most threatened groups of birds, but to date Nazca boobies are not considered at risk. These data from a well-studied species living in a relatively isolated and pristine area will provide a baseline for these birds if presented as unwell from effects such as anthropogenic stress, contaminants, or disease.

Adult boobies were sampled in a subsection of the nesting colony at Punta Cevallos (approximately 3,600 breeding pairs) known as the Mini-Area (1°23′S, 89°37′W)[3] between 17 and 24 August 2003 at least 2 mo before annual egg laying began. Blood samples were collected from 121 male and 57 female boobies (sexes are dimorphic by voice[17]). All sampled birds were known residents that had been banded in earlier years at this site. Blood sampling in these diurnal foragers was standardized to the same circadian phase (2000–2400 hours), when colony attendance is highest.[2] Because the birds, as well as all land animals, in the Galápagos evolved without large mammalian predators and are not perturbed by human presence, all birds were caught by hand at their nest site territories and restrained by hand (see Fig. 1 of Apanius et al.[3]). Blood samples (1–2 ml; $\leq$1% of body weight; Table 1) were collected by brachial venipuncture, placed in a 1.5 ml polypropylene microcentrifuge tube, allowed to clot at ambient temperature for 2–4 hr, and then centrifuged at 10,000 rpm for 10 min. Serum was then transferred to a clean 1.5 ml cryovial and frozen in the field at −70°C in a propane-powered freezer. Samples were transported from the field on dry ice in November 2003 and then stored at −80°C until laboratory analysis. Serum enzymes, metabolites, and proteins kept frozen at −70 to −80°C are stable for at least 1 yr,[5] and most are stable for 25 yr.[11] All birds present in the Mini-Area subsection of the colony were sampled and

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Table 1. Sample size (*n*), mean, SD, median, minimum (Min), and maximum (Max) values, reference interval, and 90% confidence intervals (CIs) of lower and upper reference limits (RLs) of mass and serum chemistry values measured in Nazca boobies from Isla Española, Galápagos Islands. Values of males and females are presented separately for those variables in which sex explained more variance in the data than did the null model. UA indicates uric acid; BUN, blood urea nitrogen; CRE, creatinine; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; TG, triglycerides; CHL, cholesterol; TP, total protein; and IgY, immunoglobulin Y.

Analytes	*n*	Mean	SD	Median	Min	Max	Reference interval	90% CI of RL	
								Lower	Upper
Mass (g)									
Male	115	1,669.4	1,110.8	1,650.0	1,450.0	2,000.0	1,445.6–1,884.7	1,416.7–1,477.5	1,848.8–1,916.8
Female	57	1,919.0	136.5	1,914.2	1,685.0	2,250.0	1,640.6–2,187.7	1,602.3–1,697.0	2,121.6–2,242.1
UA (μM/L)									
Low	95	297.8	36.3	293.3	235.3	388.1	221.3–365.3	210.1–231.5	351.7–378.0
High	68	585.3	122.2	573.0	400.4	917.9	396.2–878.3	376.2–415.6	802.0–952.8
BUN (mM/L)	163	6.8	3.6	6.4	1.7	28.1	2.7–15.2	1.7–3.0	13.7–28.1
CRE (μM/L)	160	43.8	14.6	43.1	11.3	85.1	13.3–76.9	11.3–21.4	69.7–85.1
AST (U/L)	163	189.4	43.8	181.3	118.8	344.0	120.0–330.6	118.8–134.0	268.2–344.0
CK (U/L)	161	1,285.2	634.1	1,091.6	563.9	3,438.5	596.7–2,941.0	563.9–623.5	2,652.2–3,438.5
LDH (U/L)									
Male	109	195.8	58.6	186.8	99.5	445.5	111.8–340.0	103.2–120.4	308.6–375.9
Female	53	181.2	59.2	173.1	100.1	338.1	99.0–336.3	90.9–108.6	290.9–392.2
TG (mM/L)	163	0.9	0.2	0.8	0.4	2.1	0.63–1.58	0.40–0.66	1.45–2.06
CHL (mM/L)	163	5.1	0.7	5.0	1.1	7.8	3.9–6.8	1.1–4.2	6.3–7.8
TP (g/L)	160	45.1	3.1	44.8	38.7	57.0	39.7–52.9	38.7–40.5	50.1–57.0
IgY (g/L)	178	6.4	2.7	6.0	2.2	26.0	2.7–11.3	2.2–3.0	10.5–26.0

all were the subjects of an ongoing behavioral observation study about mate choice[16] at the time of sampling. None of the birds had overt behavioral or physical abnormalities at the time of sampling or during the behavioral study.[16]

Serum concentrations of enzymes (aspartate aminotransferase [AST; Thermo Scientific TR70121, Waltham, Massachusetts 02451, USA], creatine kinase (CK; Diagnostic Chemicals 326-17, Waltham, Massachusetts 02451, USA], and lactate dehydrogenase (LDH; Diagnostic Chemicals 327-10), total protein (TP; TR34021, Thermo Scientific), and metabolites (uric acid [UA; TR242, Thermo Scientific], blood urea nitrogen [BUN; TL124, Thermo Scientific], creatinine [CRE; TR351, Thermo Scientific], triglycerides (TG; TL224, Thermo Scientific), and cholesterol (CHL; 234-60, Diagnostic Chemicals) were measured using clinical chemistry reagent kits. All assays were performed following manufacturers' protocols in June–July 2005. Samples were run in triplicate in 96-well microplates and absorbance was measured at the appropriate wavelength with a microplate reader (Victor3, Perkin-Elmer, Santa Clara, California 95054, USA). Hemolyzed and/or lipemic samples (n = 15) were excluded from analyses.

Circulating immunoglobulin Y (IgY) was measured using protein gel electrophoresis following a previously established protocol.[3] Briefly, serum [IgY] was measured by electrophoretic separation from other serum proteins in 7.5% polyacrylamide gels followed by quantitative staining and densitometry. Purified chicken IgY (Sigma-Aldrich Corp. I4881, St. Louis, Missouri 63103, USA) was used to construct a standard curve (2, 4, 6, 8, 10 mg/ml) in each gel. This concentration range produced a linear standard curve with $r^2 > 0.95$ for each gel.

Of the 178 birds that provided usable blood samples, weights were obtained from 115 males and 57 females with a Pesola spring scale (2,500 ± 20 g) from 23 August to 30 September 2003. Weights were measured from 0200 to 0600 hours (at a different point in the circadian cycle than blood sampling; see above), when weight of ingested food was lowest, to best estimate body weight.[2]

Data were examined for outliers using the Dixon outlier range statistic, which identifies the most extreme value at the upper or lower limit as an outlier if $D/R > 0.3$, where $D = |\text{extreme value} - \text{next nearest value}|$ and R is the range of all values.[6] Extreme values were removed and data

Table 2. Body weights and serum chemistry values previously reported in the literature for Sulid species.[a]

	n	Mean ± SD	Min	Max
Brown booby (*Sula leucogaster*)[21]				
Male				
Mass (g)	35	1,200 ± 100	1,000	1,400
TP (g/L)	16	38 ± 7	25	52
ALB (g/L)	16	15 ± 2	11	21
Glob (g/L)	16	22 ± 4	14	31
AST (U/L)	16	320 ± 83	204	481
CK (U/L)	16	427 ± 221	131	782
UA (μM/L)	16	993.3 ± 672.1	237.9	2,319.7
Female				
Mass (g)	35	1,500 ± 100	1,300	1,700
TP (g/L)	17	40 ± 5	31	49
ALB (g/L)	17	16 ± 2	12	20
Glob (g/L)	17	24 ± 3	18	31
AST (U/L)	17	306 ± 129	151	690
CK (U/L)	17	774 ± 361	172	1,743
UA (μM/L)	17	1,219.3 ± 547.2	571.0	2,450.6
Red-footed booby (*Sula sula*), sexes combined[20]				
Mass (g)	34	1,000 ± 100	900	1,200
TP (g/L)	32	27 ± 3	21	31
ALB (g/L)	31	12 ± 1	10	15
Glob (g/L)	31	16 ± 2	12	19
AST (U/L)	32	302 ± 150	134	922
CK (U/L)	32	341 ± 210	22	937
UA (μM/L)	32	963.6 ± 523.4	178.4	1,945.0
Red-footed booby (*S. sula*), sexes combined[18]				
TP (g/L)	21	35.6 ± 3.0		
ALB (g/L)	21	11.7 ± 1.0		
Glob (g/L)	21	20.0 ± 9.0		
AST (U/L)	21	465.8 ± 181.1		
CK (U/L)	21	940.1 ± 371.4		
UA (μM/L)	21	648.3 ± 487.7		
Nazca booby (*Sula granti*), sexes combined[18]				
TP (g/L)	24	37.6 ± 4.0		
ALB (g/L)	24	12.2 ± 1.0		
Glob (g/L)	24	24.0 ± 6.0		
AST (U/L)	24	310.3 ± 141.7		
CK (U/L)	24	871.8 ± 271.1		
UA (μM/L)	24	719.7 ± 428.3		

[a] Min indicates minimum; Max, maximum; TP, total protein; ALB, albumin; Glob, globulin; AST, aspartate aminotransferase; CK, creatine kinase; and UA, uric acid.

were reexamined until no more extreme values were identified.

Differences in foraging success could be reflected in serum chemistry values, especially in carnivorous birds,[9] so a frequency distribution of the natural log of each variable was examined for bimodality (successful vs unsuccessful foragers).[7] Bimodal distributions were separated into high and low groups before further analyses were performed.

Sex differences within variables were assessed using general linear models (some distributions required log transformation to meet normality assumptions). The Akaike information criterion corrected for small sample sizes (AICc) was used to assess explanatory value of sex over the null model that excluded the effect.[4] Sex-specific values were reported if the model that included sex was the top model as indicated by AICc values. Dell Statistica (version 13, Dell, Inc, Tulsa, Oklahoma 74104, USA) was used for all statistical analyses.

Reference intervals of each variable were determined using Reference Value Advisor[10] and are

presented as the 2.5th and 97.5th percentiles. When sample size permitted ($n \geq 120$), nonparametric methods were used to estimate reference intervals and 90% confidence intervals (CIs) of reference limits. For sample sizes between 20 and 120, the reference interval and 90% CI of reference limits were estimated using a robust parametric method.[12]

Extreme values were found in CK (one high and one low), LDH (males: one high), and TP (two high and one low), and were excluded from all analyses. Only UA had a bimodal distribution. All serum chemistry values and body weight data are collated in Table 1. Males and females differed in mass (effect size [R^2] = 50.3) and LDH, but the effect size was relatively small for LDH ($R^2 = 2.2$; see Supplemental Table 1 for all model comparisons). Females were heavier than males, as expected for sulids.[17] Body mass did not correlate significantly with any serum chemistry value. UA was the only bimodal variable, which probably reflects differences in recent foraging success in overtly healthy birds, because UA is elevated in carnivorous birds shortly after feeding.[9]

Data from other studies of Nazca boobies and other sulids are collated in Table 2 to provide this information in one location for future reference. Variability of UA was high in previously sampled sulids.[18,20,21] The birds in the study were split into two groups (low and high), which lowered the overall variability of the dataset. The large mean UA values in the previous studies are likely influenced by high values in the dataset. AST and CK in this study were similar to those from Nazca boobies elsewhere in the Galápagos[18] and in other sulid species.[18,20,21]

This study reports baseline serum chemistry reference intervals for a free-ranging sulid species, the Nazca booby, for the first time. These data are critical for practitioners investigating responses of seabirds to exposures to pollutants or toxic algal blooms, coastal restoration efforts, food shortages, or emerging diseases. These data from a wild species living in a relatively isolated and pristine area provide valuable baseline data for the species.

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