Hematologic, plasma protein, and biochemical profiles of brown pelicans (Pelecanus occidentalis)

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Objective—To establish hematologic and biochemical reference values for the brown pelican (Pelecanus occidentalis).

Animals—31 captive, healthy, but permanently disabled pelicans and 35 wild-caught, healthy pelicans from a rehabilitation facility on the east coast of Florida.

Procedures—Samples of venous blood were collected from each pelican, and hematologic, plasma biochemical, and electrophoretic protein analyses were performed. Student t-tests were used to compare blood values between captive versus wild-caught, adult male versus adult female, and adult versus juvenile pelicans.

Results—Hematologic and electrophoretic values were similar between male and female, adult and juvenile, and captive and wild-caught pelicans. Significant sex differences existed for plasma calcium and triglyceride concentrations. Plasma concentrations of calcium, cholesterol, and CO₂ content differed between captive and wild-caught adults. No significant differences were found between wild-caught adult and juvenile pelicans.

Conclusions and Clinical Relevance—Our plasma biochemical results are similar to those of other brown pelicans and confamilial species. Additional studies on seabirds are encouraged, as age, sex, reproductive status, feeding habits, and captivity are important variables for health assessment in this and other aquatic species. (Am J Vet Res 2000;61:771–774)

Brown pelicans (Pelecanus occidentalis) are common residents along the southeastern and west coasts of the United States, as well as Mexico, the West Indies, Caribbean, and South America.¹⁻³ Brown pelicans nest and roost in colonies on small coastal islands. In 1970, as the result of residues of dichlorodiphenyltrichloroethane (DDT) and related compounds, pelican reproductive success declined precipitously, forcing the inclusion of the brown pelican on the Endangered Species List. After the ban on the use of DDT and related compounds, the pelican populations rebounded to the point where the populations on the Atlantic coast, Florida, and Alabama have been upgraded to threatened species whereas remaining southeast coastal, Caribbean, and west coast populations remain endangered.⁴⁻⁵ Other threats to brown pelicans include human disturbance of breeding, chemical and oil spills, human and natural toxins, and primarily, entanglement in fishing lines, hooks, and sinkers of careless fishermen. The US Fish and Wildlife Service continually monitor brown pelican populations.

Two factors contribute to the increasing numbers of brown pelicans arriving at rehabilitation facilities and as captive residents at marine parks, aquaria, and zoos. The first are injuries caused by fishing lines and hooks. Many rehabilitation facilities rescue and treat broken limbs and infections caused by these accidents. Second, the trend toward maintaining natural ecosystems as a means of species conservation has, and likely will continue to, resulted in increasing numbers of all seabird species kept in zoos and marine parks. In attempts to reconstruct natural ecosystems, pelicans will likely be integral residents, as they adapt easily to and thrive in captivity.

Compared with psittacines, poultry, and select other high profile species in captivity and in the wild, physiologic data on individual seabird species are relatively scarce but increasing.⁶⁻¹⁰ As seabirds are increasingly coming under veterinary attention, accurate clinicopathologic data are required for adequate health assessment. The aim of this study was to obtain blood samples from the resident population of brown pelicans in a rehabilitation center and the surrounding area of south-east Florida and compile and expand upon previous physiologic data for health assessment of this species.

Materials and Methods

Study population—The Pelican Harbor Seabird Station is located on Biscayne Bay, Miami, Florida. The seabird station rescues and rehabilitates all types of seabirds but primarily focuses on pelicans. Resident at the station were > 35 healthy but permanently flightless pelicans. The pelicans were flightless because of sustained injuries, or because they are missing a wing. These injuries resulted from accidental entanglement with fishing lines or hooks. Blood samples were obtained from 31 resident pelicans of this population in October and November 1998 to establish reference range values for hematologic, plasma protein electrophoresis, and biochemical analyses.

The 31 birds (17 males and 14 females) were housed in 2 separate pens, each with a small shallow pool of water. Pelicans were fed 0.5 to 0.75 kg of mackerel or herring twice daily. Cages were cleaned and fresh water pools filled once daily. These pelicans had been housed together, bred successfully, and been clinically normal (ie, no signs of disease, no changes in appetite, no parasitic diseases, etc) for several (3 to 7) years.¹¹ Sex of the captive birds was determined by bill measurements,¹² behavioral observations of mating, and independently corroborated by nucleic acid amplification performed on whole blood in EDTA at the Infectious and Disease Laboratory, University of Georgia.¹³

Additional healthy adult (n = 14) and juvenile (21) brown pelicans were randomly captured by hand, using bait.

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Age was assigned on the basis of plumage characteristics. The criterion used to determine health in these birds included assessment of general body condition (by palpation of keel musculature) and feathering, lack of overt clinical signs of diseases (i.e., no nasal or ocular discharge, clear eyes, normal appearing feces, etc), normal amount of activity, and lack of external lesions. Blood samples were obtained from these birds in December 1998 and January 1999, and represent the wild-caught population of birds.

Analyses of blood samples—Venous blood (1 to 2 ml collected by 1-ml syringes with 25-gauge needles) was obtained from either the medial metatarsal or cutaneous ulnar veins. Samples were evacuated into small plastic tubes containing EDTA as the anticoagulant (for hematologic evaluation) and lithium heparin (for biochemical analysis). Samples were refrigerated (4°C) and analyzed within 36 hours of collection. Hematologic evaluation was performed manually and included RBC count, WBC count, hematocrit, WBC differential, and presence or absence of hemoparasites.

Measured plasma biochemical variables included glucose, sodium, potassium, blood urea nitrogen (BUN), creatinine, calcium, phosphorus, uric acid, cholesterol, and triglyceride concentrations, CO₂ content, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LD), γ-glutamyltransferase (GGT), creatinine kinase (CK), amylase, and lipase activities.

Agarose gel electrophoreses were run as described previously. Briefly, 1:4 diluted microsamples (3 to 5 μl) were loaded onto the gel. The gel was electrophoresed for 40 minutes at 100 V, dried, stained with Paragon Blue, and scanned with a densitometer at 600 nm. Percentages and absolute values (g/dl) for the protein fractions were then determined on the basis of the total protein obtained by refractometry.

Statistical analyses—Data from the following 4 groups of brown pelicans were compared: captive males, captive females, wild-caught adults (of both sexes), and wild-caught juveniles (of both sexes). Wild-caught juveniles included birds not in adult plumage ranging from 1 to 3 years old. Mean (± SD) values are presented. Means between captive males and females, captive and wild-caught adults, and wild-caught adults and juveniles were compared by Student t-tests. Because of the large number of statistical comparisons, the P value for significance was systematically reduced to P < 0.001 as previously described for the analysis of tabulated data.

Results
There were no significant differences between captive adult males and females, between captive and wild-caught adults, or between wild-caught adults and juveniles. Combined mean hematologic values (n = 44; i.e., captive and wild, adult and juvenile, male and female pelicans) were as follows: 2.66 ± 0.4 × 10³ RBC/μl, 16.7 ± 4.7 × 10³ WBC/μl, 8.3 ± 2.1 × 10³ heterophils/μl, 7.3 ± 2.2 × 10³ lymphocytes/μl, 0.87 ± 1.0 × 10³ γ-globulin.

| Table 1—Mean (± SD) plasma biochemical values of captive and wild brown pelicans |
|---------------------------------|-------------|-------------|-------------|-------------|
| Variables                      | Captive     | Wild        |             |
|                                | Males       | Females     | Both        | Adults      | Juveniles   |
| Glucose (mg/dl)                | 216 ± 32 (n = 17) | 226 ± 22 (n = 14) | 220 ± 28 (n = 31) | 162 ± 23 (n = 7) | 188 ± 74 (n = 9) |
| Sodium (mM/L)                  | 148 ± 3 (n = 17)  | 146 ± 2 (n = 14)  | 147 ± 3 (n = 31)  | 144 ± 4 (n = 7)  | 149 ± 43 (n = 9) |
| Potassium (mM/L)               | 5.3 ± 0.7 (n = 17) | 5 ± 1 (n = 14)    | 5.1 ± 0.8 (n = 31) | 5.7 ± 1 (n = 7)  | 7.3 ± 5.6 (n = 9) |
| CO₂ (mM/L)                     | 19 ± 2 (n = 17)   | 18 ± 3 (n = 14)   | 18 ± 3 (n = 31)   | 23 ± 2 (n = 7)   | 24 ± 4 (n = 9)   |
| BUN (mg/dl)                    | 4.4 ± 0.6 (n = 17) | 4.1 ± 0.9 (n = 14) | 4.3 ± 0.7 (n = 31) | 5.9 ± 2.5 (n = 7) | 8.4 ± 4.8 (n = 9) |
| Creatinine (mg/dl)             | 0.7 ± 0.2 (n = 16) | 0.6 ± 0.2 (n = 12) | 0.7 ± 0.2 (n = 28) | 0.9 ± 0.4 (n = 10) | 0.8 ± 0.2 (n = 9) |
| Total protein (g/dl)           | 4.3 ± 0.7 (n = 17) | 6.0 ± 1.9 (n = 14) | 5.1 ± 1.6 (n = 31) | 5.1 ± 1.2 (n = 11) | 4.3 ± 0.7 (n = 12) |
| Calcium (mg/dl)                | 9.6 ± 0.5 (n = 17) | 15.4 ± 3.3 (n = 14) | 12.4 ± 3.5 (n = 31) | 9.8 ± 0.7 (n = 7) | 9.5 ± 1.2 (n = 9) |
| Phosphorus (mg/dl)             | 5.6 ± 0.9 (n = 17) | 7.4 ± 3.4 (n = 14) | 6.4 ± 2.5 (n = 31) | 5.5 ± 1.1 (n = 10) | 5.6 ± 1.1 (n = 12) |
| Uric acid (mg/dl)              | 13.6 ± 7 (n = 17)  | 10.6 ± 5 (n = 14)  | 12.3 ± 7 (n = 31)  | 16.9 ± 7 (n = 10) | 13.5 ± 7 (n = 7)  |
| ALT (IU/L)                     | 50 ± 19 (n = 17)   | 39 ± 14 (n = 14)   | 45 ± 18 (n = 31)   | 50 ± 30 (n = 11)  | 47 ± 27 (n = 12)  |
| AST (IU/L)                     | 318 ± 66 (n = 17)  | 247 ± 120 (n = 14) | 286 ± 100 (n = 31) | 307 ± 131 (n = 11) | 276 ± 132 (n = 11) |
| LDH (IU/L)                     | 2,734 ± 707 (n = 12) | 2,001 ± 496 (n = 13) | 2,352 ± 701 (n = 25) | 3,837 ± 2,280 (n = 7) | 3,812 ± 1,033 (n = 9) |
| GGT (IU/L)                     | 11.5 ± 2 (n = 17)   | 12.7 ± 3 (n = 14)   | 12 ± 3 (n = 31)    | 11.5 ± 3 (n = 11)  | 10.6 ± 2 (n = 11)  |
| Cholesterol (mg/dl)            | 144 ± 19 (n = 17)   | 190 ± 55 (n = 14)   | 165 ± 49 (n = 31)   | 113 ± 12 (n = 7)   | 917 ± 27 (n = 9)   |
| Triglycerides (IU)             | 51 ± 39 (n = 17)    | 587 ± 418 (n = 13)  | 283 ± 383 (n = 30)  | 67 ± 61 (n = 7)    | 58 ± 34 (n = 9)    |
| CK (IU/L)                      | 998 ± 285 (n = 16)  | 961 ± 754 (n = 14)  | 981 ± 545 (n = 30)  | 1,229 ± 694 (n = 11) | 1,187 ± 360 (n = 10) |
| Amylase (IU/L)                 | 1,004 ± 99 (n = 17) | 981 ± 143 (n = 14)  | 993 ± 119 (n = 31)  | 1,106 ± 82 (n = 7)  | 989 ± 132 (n = 9)  |
| Lipase (IU/L)                  | 48 ± 16 (n = 17)    | 52 ± 29 (n = 14)    | 49 ± 22 (n = 31)    | 40 ± 18 (n = 11)   | 54 ± 44 (n = 12)   |

BUN = Blood urea nitrogen. AST = Aspartate aminotransferase. LD = Lactate dehydrogenase. GGT = Gamma glutamyltransferase. CK = Creatine kinase.

Indicates a significant (P < 0.001) difference between the sexes. Indicates a significant (P < 0.001) difference between captive and wild-caught adults (both sexes combined).
centrations. Significant differences in plasma CO2 concentration were found for plasma calcium and triglyceride concentrations when pelicans are maintained with salt water pools and wild-caught birds. Other variables for which Wolf's values differed from those obtained in our study include CO2 content, amylase, ALT, AST, and LD activities, and uric acid and triglyceride concentrations. Reasons for these differences may include sample type (eg, use of plasma versus serum), intrinsic population differences, or differences in study design. In Wolf's study, the number of birds in each age group ranged from 3 to 14, blood samples were obtained from these birds monthly, and all values were used to calculate mean values. Overall, however, there was general concurrence between our results and those of Wolf et al. Substantial variation exists and factors such as captivity, sex, age, reproductive and nutritional status, and feeding should be considered when using reference ranges for health assessment in this and other species.

There are several differences in the protein electrophoretic pattern of pelicans as compared with many other avian species; however, the general pattern is similar. Balasch et al performed protein electrophoresis from blood samples of 6 pelicans of 2 different subspecies. Albumin and γ-globulin fractions are similar between our study and theirs; however, we saw no prealbumin fraction, and there were substantial differences in the α- and β-globulin fractions. These differences may reflect different gel electrophoretic techniques and equipment and subsequent variable protein fractionation.

Several comparisons can be drawn between the biochemical values obtained in our study versus those obtained by Wolf et al in their study of brown pelicans on the west coast of Florida and other confamilial species. Plasma calcium and triglyceride concentrations were significantly higher in females than in males in our study. These results are likely the result of pre-egg producing physiologic adaptations. Similar results are found in great frigatebirds. Wolf et al found greater serum calcium concentrations in egg producing versus non-egg-producing females; however, they made no comparisons for blood calcium concentrations between the sexes.

In our study, captive adult pelicans had greater plasma calcium and cholesterol concentrations than wild-caught adults. These differences may be reflective of feeding and metabolic differences as blood samples were obtained from captive birds usually in the middle to late morning, approximately 2 to 4 hours after the morning feeding, whereas the timing of last feeding in the wild birds was likely not that regular. In addition, the sexes of the wild-caught adults were not ascertained and it is unknown whether the wild group of pelicans was male biased and whether this may have contributed to the difference in plasma calcium concentration.

Wolf et al found higher serum sodium concentrations when pelicans are maintained with salt water pools than with fresh water pools. Our data do not support their findings as no statistical difference was seen in our study between birds maintained with fresh water pools and wild-caught birds. Other variables for which Wolf's values differed from those obtained in our study include CO2 content, amylase, ALT, AST, and LD activities, and uric acid and triglyceride concentrations. Reasons for these differences may include sample type (eg, use of plasma versus serum), intrinsic population differences, or differences in study design. In Wolf's study, the number of birds in each age group ranged from 3 to 14, blood samples were obtained from these birds monthly, and all values were used to calculate mean values. Overall, however, there was general concurrence between our results and those of Wolf et al. Substantial variation exists and factors such as captivity, sex, age, reproductive and nutritional status, and feeding should be considered when using reference ranges for health assessment in this and other species.

Discussion
To our knowledge, our study provides the most comprehensive investigation on hematologic variables of brown pelicans in North America to date. In general, there is good agreement between the published values for several aquatic birds (including pelicans from Spain) and brown pelicans in our study. Variation in hematologic variables seems to be centered on leukocyte count and heterophil-to-lymphocyte ratio. White storks, white pelicans, and great frigatebirds have unequal ratios, often up to 3 to 4:1, whereas brown pelicans and Chilean flamingos as well as most psittacines have heterophil-to-lymphocyte ratios of 1.5 to 1.1. Albumin comprises the major portion of serum protein. Globulins are subdivided into the acute phase reactants α1-,

α2- and β-globulins and the immunoglobulins or γ-globulins. In our study, we used plasma for electrophoresis. In our experience, there are negligible differences in electrophoretic patterns when using plasma versus serum.

Protein electrophoresis patterns are very useful for health and disease assessment. Significant differences in plasma calcium and triglyceride concentrations were found for plasma calcium and triglyceride concentrations.
Results of our study expand upon previous information on the brown pelican. More baseline information is needed on all aquatic avian species as more are being cared for at rehabilitation facilities. Many of these species live and forage in inshore coastal waters, and as such can be considered as potential sentinels for oceanic and environmental hazards and diseases that can ultimately impact other aquatic species as well as humans.

References