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Serum chemistry values in wild black vultures in Mississippi, USA

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Abstract: Vultures (Cathartidae and Accipitridae) play an important role in ecosystem balance by rapidly disposing animal carcasses and thus preventing the potential spread of pathogens. Blood chemistry values provide a means of assessing the health of wildlife and wild animal populations; however, there are significant differences in chemistries among species and when comparing captive and free-living New and Old World vultures. In 2007, we collected blood serum from 30 female and 14 male wild, healthy black vultures (*Coragyps atratus*) live-trapped by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services from a power substation in Lowndes County, Mississippi, USA. We analyzed the blood serum to provide serum chemistry base values for use in clinical pathology. The chemical analytes we measured included sodium, chloride, potassium, carbon dioxide, anion gap, glucose, creatinine, calcium, phosphorus, total protein, albumin, globulin, and aspartate aminotransferase. In general, blood chemistry values of black vultures were similar to those found in New and Old World vultures and raptor species. Average chemistry values for males were lower than females for sodium, chloride, creatinine, calcium, total protein, albumin, and globulin. The serum chemistry values we describe in this paper can be important indicators of avian health by gender for the black vulture. Our study provided important blood chemistry values from a large sample size, which is rarely available in free-ranging black vultures. These values could be used by scientists, veterinary pathologists, wildlife rehabilitation centers, and other researchers for baseline data for wild and free-ranging birds. Furthermore, the use of such parameters in assessing population health may enable conservationists to further research environmental conditions affecting species reproduction and survival.

Key words: avian health, black vulture, clinical pathology, *Coragyps atratus*, Mississippi, serum chemistry, sex differences, species conservation, raptors, wildlife rehabilitation

Blood chemistry values provide a means of assessing the health of wildlife and wild animal populations (Campbell and Dein 1984). While Cooper (1998) provided serum chemistry values for all birds of prey in general, Heidenreich (1997) noted significant differences in chemistries among species. Villegas et al. (2002) and Dobado-Berrios et al. (1998) reported that blood chemistry parameters differed between wild or free-living Egyptian vultures (*Neophron percnopterus*), when compared to captive birds.

Vultures (Cathartidae and Accipitridae) are an important group for their ecological role in rapidly disposing of animal carcasses, preventing potential dissemination of pathogens, attracting other scavengers to the carcasses, and cycling nutrients (Beasley et al. 2015, Inger et al. 2016). In many regions of the world, vulture popula-

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Figure 1. Black vulture (*Coragyps atratus*) perched on a utility pole (*photo courtesy of U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center*).

tions are declining. Currently, the Switzerland-based International Union for the Conservation of Nature (IUCN) lists 15 out of 22 species of vultures as near threatened, endangered, or critically endangered (IUCN 2018). India's populations of white-backed vultures (*Gyps bengalenisi*), long-billed vultures (*G. indicus*), slender-billed vultures (*G. tenuirostris*), and red-headed vultures (*Sarcogyps clavus*) have decreased 99% from 1993 to 2002 due to the toxicity to non-steroid anti-inflammatory drugs (NSAIDs) diclofenac and ketoprofen (Shultz et al. 2004; Cuthbert et al. 2007; Naidoo et al. 2010*a*, *b*). Vultures exposed to NSAIDs when feeding on the carcasses of livestock treated with NSAIDs die within a few days from renal failure, liver injury, and diffuse visceral gout (Oaks et al. 2004, Swan et al. 2006). As a result, India has banned the use of diclofenac and ketoprofen in veterinary medicine. Despite this ban, many of the carcasses of *Gyps* vultures that were collected from July 2000 to April 2012 were still positive for diclofenac residues in liver and/or kidney tissues, and visceral gout was identified in all carcasses (Cuthbert et al. 2016).

Free-living black vultures (*Coragyps atratus*; Figure 1) are opportunistic when feeding, and their diet may include other animals, livestock, fish, invertebrates, and carrion, and they can be found feeding at landfills, dumpsters, zoos, or anywhere food is available (Avery and Lowney 2016). Given increased levels of total protein, increased urate, creatinine, and albumin found in captive birds may indicate that they are fed diets containing protein in excess of their requirements (Dobado-Berrios et al. 1998). Captive adult vultures have significantly higher levels of total protein, albumin, creatinine, urate, cholesterol, calcium, phosphorus, and aspartate aminotransferase (AST) when compared to wild birds. The investigators speculated that these differences can result due to diet quality and reduced physical activity of captive Egyptian vultures. Due to these differences, comparing values from captive and wild birds should be carefully considered (Dobado-Berrios et al. 1998). The limited information available from captive zoo populations may not be an accurate quality indicator of blood parameters because of altered diets, activity levels, age, nutrition, and health status in captivity (Perry et al. 1986, Ferrer et al. 1987, Dobado-Berrios et al. 1998). One problem when assessing the health of wild populations is the fact that limited data are available on normal clinical biochemistry values for most species of vultures.

Black vultures are a common scavenger of the southeastern and eastern United States (United States Department of Agriculture [USDA], Animal and Plant Health Inspection Service, Wildlife Services [WS] 2019). While some data exist regarding packed cell volumes and protein concentrations (Coleman et al. 1988), little research has been conducted on blood chemistry values of black vultures. The objectives of our study were to: (1) determine baseline blood chemistry values, and (2) determine blood chemistry values for each sex of adult black vultures in Mississippi, USA.

Study area

The black vultures we studied were live-trapped by the USDA WS from a power substation in Lowndes County, Mississippi, along the Tennessee Tombigbee waterway, which forms a border between Mississippi and Alabama, USA (Figure 2). Mississippi is in the southeastern United States at -88.82 decimal degrees longitude and 33.45 decimal degrees latitude. The elevation in the area of live-trapping is 102 m above sea level. The average monthly temperatures in the area range from a high of 12.2°C and a low of 2.2°C in January to a high of 33.3°C and a low of 22.2°C in July. Average monthly rainfall ranges from a low of 7 cm in August to a high of 13 cm in February with an average

Figure 2. Study area in Lowndes County, Mississippi, USA (*photo from Bing images*).

annual rainfall of 141 cm. Lowndes county is dominated by 2 major physiographic regions: the Fall Line Hills (Tennessee-Tombigbee River Hills) and the Black Prairie belt. The Fall Line Hills is characterized by rugged, hilly topography covered mostly by bottomland and upland hardwood forests. The Black Prairie Belt consists mainly of gently rolling hills of low relief and has been predominantly developed into agricultural production (Natural Resources Conservation Service 1979).

Methods

To conduct our study, we examined 30 female and 14 male adult wild black vultures that were removed from a power substation in Lowndes County, Mississippi between March and May 2007 by WS during damage management operations to reduce vulture populations and mitigate their damage. The black vultures were live-trapped using a modified walk-in corral trap baited with road-killed animals.

Live-trapped birds were placed in cages and transported to the College of Veterinary Medicine (CVM) at Mississippi State University for gross examination and obtaining blood samples. Following phlebotomy, the birds were humanely euthanized according to standard protocols and necropsied. Gender was determined during the postmortem examination. All 44 birds were considered at least 12 months old based on estimates from physical characteristics such as breeding phenology and growth development of the species (Buckley 1999). More accurate methods of determining age were not yet available, and we could not classify the birds based on same year age class or older. Black vultures nest in mid-February to early April in the southern United States with peak chick hatch mid-March to early May and fledglings observed from early June through early August. Because the samples were collected during March through May, vultures were adult size and we determined that the birds were at least after hatch year.

Blood samples

We obtained blood samples from a brachial wing vein using 20-gauge needles and 10-cc syringes. Blood samples were submitted to the CVM diagnostic laboratory for analysis. The blood was allowed to clot and spun at 3,000 rpm for 7 minutes. Serum was removed by individual transfer pipettes and placed into a plastic transfer tube and labeled with sample identification. Samples were then immediately loaded into the chemistry analyzer. If processing was not immediately possible, serum was refrigerated at 2–8°C for up to 24 hours.

Serum chemistry

We ran the serum chemistry tests on the Alera® system by Alfa Wassermann Diagnostic Technologies (West Caldwell, New Jersey, USA). The electrolytes (Na, K, and Cl) were measured using ion selective electrodes. All other chemistry tests were measured on the Alera using reagents manufactured by Alfa Wassermann. Globulin values were calculated as the difference of the total protein and albumin concentrations.

Statistics

We calculated the mean, confidence interval (CI), and range of each serum chemistry parameter by sex. Because the data were normally distributed, we tested for differences between male and female wild black vultures using an unpaired *t*-test for normally distributed data. Significance values were set at $P \leq 0.05$ for all tests.

Results

On gross physical exam, all birds appeared to be in good health with no abnormalities or evidence of disease. We determined the range of values for 13 blood parameters on the chemistry panel including sodium, chloride, potassium, carbon dioxide, anion gap, glucose, creatinine, calcium, phosphorus, total protein, albumin, globulin, and aspartate aminotransferase (Table 1). Male and female vultures differed in 7 of the 13 parameters (Table 2). Male

Table 1. Serum chemistry values of black vultures (*Coragyps atratus*) in Mississippi, USA, 2007, males and females combined. Asterisk (*) denotes significant ($P < 0.05$) sex differences in serum chemistry. AST = aspartate aminotransferase.

	All birds			
	Lower CI	Mean	Upper CI	*n*
*Sodium (mEq/L)	154.61	155.84	157.06	44
Potassium (mmol/L)	1.96	2.23	2.49	27
*Chloride (mmol/L)	118.19	119.30	120.40	44
Carbon dioxide (mEq/L)	21.75	22.52	23.28	43
Anion gap (mEg/L)	14.57	15.67	16.76	27
Glucose (mg/dL)	214.62	228.19	241.76	43
*Creatinine (mg/dl)	0.30	0.32	0.34	43
*Calcium (mg/dl)	8.68	8.91	9.14	43
Phosphorus (mg/dl)	1.69	1.92	2.15	43
*Total protein (g/dl)	3.50	3.64	3.78	43
*Albumin (g/dl)	1.09	1.12	1.16	43
*Globulin (g/dl)	2.40	2.51	2.63	43
AST (UI/L)	106.96	204.49	302.01	43

Table 2. Serum chemistry values by sex for wild black vultures (*Coragyps atratus*) in Mississippi, USA, 2007. AST = aspartate aminotransferase.

	Male				Female				*t*-test	
	Lower CI	Mean	Upper CI	*n*	Lower CI	Mean	Upper CI	*n*	*t*	*P*
Sodium	150.69	153.36	156.02	14	155.82	157.00	158.18	30	3.06	0.004
Potassium	1.82	2.46	3.10	7	1.84	2.15	2.45	20	-1.07	0.30
Chloride	115.23	117.57	119.91	14	118.92	120.10	121.28	30	2.25	0.03
Carbon dioxide	21.32	23.08	24.83	13	21.41	22.27	23.14	30	-0.97	0.34
Anion gap	11.55	14.00	16.45	7	15.03	16.25	17.47	20	1.95	0.06
Glucose	194.97	213.85	232.72	13	216.57	234.40	252.23	30	1.42	0.16
Creatinine	0.25	0.28	0.30	13	0.31	0.33	0.36	30	3.02	0.004
Calcium	7.91	8.38	8.85	13	8.92	9.14	9.37	30	3.50	0.001
Phosphorus	1.40	1.96	2.52	13	1.65	1.90	2.15	30	0.25	0.81
Total protein	3.20	3.41	3.61	13	3.57	3.74	3.92	30	2.34	0.03
Albumin	1.02	1.07	1.12	13	1.11	1.15	1.19	30	2.35	0.02
Globulin	2.14	2.32	2.51	13	2.45	2.60	2.74	30	2.23	0.03
AST	29.01	349.15	669.30	13	94.78	141.80	188.82	30	1.39	0.19

chemistry values were significantly lower than females for sodium chloride, creatinine, calcium, total protein, albumin, and globulin (Table 2). Potassium, carbon dioxide, anion gap, glucose, phosphorus, and AST levels did not differ between the sexes.

Discussion

Very few chemistry values exist for wild black vultures, and this study provided important information on the range of values for free-flying adult birds. Blood chemistry values for wild black vultures have not been previously reported differentiated by sex.

Because total protein (TP) values have been used as a general indicator of physical condition in birds (de le Court et al. 1995, Dawson and Bortolotti 1997), our results provided a baseline for black vultures. In contrast to Coleman et al. (1988), which suggested minimal sex differences, we found TP levels were significantly lower in male black vultures than females (Table 2). Our results are lower than the mean values reported by Coleman et al. (1988). Vultures in this study and in Coleman et al. (1988) appeared to be healthy and showed little variation in TP; therefore, this difference may indicate geographic variation.

Mean glucose levels for black vultures with sexes combined were lower than those found in wild-caught red-tailed hawks (*Buteo jamaicensis*) and American kestrels (*Falco sparverius*) in California, USA (Stein et al. 1998). These authors also concluded that glucose levels determined from the wild hawks were higher than those previously reported in captive hawk populations. Villegas et al. (2002) reported much higher glucose levels in adult birds than we found in this study but were similar to levels in nestlings, which may be attributed to the higher rate of gluconeogenesis in adults. However, as blood glucose levels are highly influenced by diet and stress, such differences may also be explained by recent meals and stress induced by handling and capture. Differences in age, migratory status, diet, geographical location, and captive versus wild status could significantly impact blood chemistry values and need to be studied further to quantify these differences.

The calcium levels we recorded in male black vultures were lower than those of females (Table 2). While the reproductive status of the female black vultures could not be determined, an increased serum albumin in laying birds can result in inflated total serum calcium levels, which could explain the sex differences. However, in this study, albumin levels in male and female black vultures were similar, and the overall mean was lower than what has been reported in other species. Similar to other parameters, serum calcium levels are highly and acutely dependent upon diet. Therefore, the composition and availability of recent meals may explain the species differences that have been reported; when comparing free-living and captive vultures, the captive birds had significantly higher calcium levels (Dobado-Berrios et al. 1998). Globulin levels in male black vultures were slightly lower than that of females. Elevated AST levels can be indicative of muscle or kidney damage; the distribution of AST in tissues among species has been reported to be highly variable (Lewandowski et al. 1986).

The results of this study indicated that males and females may have very different serum chemistry values for some parameters. Therefore, combining genders may not provide an accurate range of serum chemistries. This contrasts with a study that found no differences in serum chemistries in male and female golden eagles (*Aquila chrysaetos*; Nazifi et al. 2008). However, that study only examined 21 golden eagles, which may not have provided a big enough sample size to detect significant differences.

Villegas et al. (2002) reported significant differences in glucose, uric acid, total protein, alkaline phosphatases, phosphorus, and AST between adults and same year age Eurasian black vultures (*Aegypius monachus*) in Spain, with nestlings having higher glucose and phosphorus values than the adults. Similarly, Dobado-Berrios et al. (1998) found significant chemistry changes in Egyptian vultures, from nestling to sub-adult and then to adult. Such changes may be important in assessing the health of captive breeding and captive-reared populations of birds, and therefore, further studies are needed to determine if age differences in serum chemistry values exist in the black vulture. While reliable non-invasive indicators of age are not available, studies have been performed examining skin biopsies to estimate the age of black vultures (Cooey et al. 2010), which may allow a more accurate comparison of age differences in future studies.

Numerous studies have indicated that captive bird blood chemistry values do not match free-ranging birds (Perry et al. 1986, Ferrer et al. 1987, Dobado-Berrios et al. 1998). However, very few chemistry values exist for black vultures, and this study provides important serum range values for free-flying adult birds. Until recently, limited data have been available on blood values by gender or species of wild bird populations. Serum chemistry values that can be important indicators of avian health have been described here by gender for the black vulture. Similar uses of blood in ecology and conservation science studies have been performed in wildlife vertebrates (Dobado-Berrios et al. 1998).

Overall, blood chemistry values we reported in this study are similar to those described for other vulture and raptor species. However, black vultures had lower values of creatinine and glucose than other species. Blood chemistry values for black vultures have not been previously reported differentiated by sex.

Management implications

This study provides important blood chemistry values from a large sample size, which is rarely available in free-ranging birds. These values could be used by scientists, veterinary pathologists, wildlife rehabilitation centers, and other researchers for baseline data for wild and free ranging birds. Furthermore, the use of such parameters in assessing population health may enable conservationists to further research environmental conditions affecting species reproduction and survival. Similar uses of blood in ecology and conservation science studies have been performed in wildlife vertebrates.

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