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Blood Serum Chemistry of Wild Alaskan Black-capped Chickadees (*Poecile atricapillus*) with Avian Keratin Disorder

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ABSTRACT: We measured serum chemistries in wild Black-capped Chickadees (*Poecile atricapillus*) from Alaska to test for potential differences associated with beak deformities characteristic of avian keratin disorder. Lower uric acid in affected birds was the only difference detected between groups, although sample sizes were small. This difference could be associated with fasting or malnutrition in birds with beak deformities, but it is challenging to interpret its biologic significance without reference values. Black-capped Chickadees had high levels of aspartate aminotransferase, lactate dehydrogenase, and creatine kinase relative to reference values for companion birds. However, all serum chemistry parameters from our study were within the range of values reported from other apparently healthy wild-caught birds.

Key words: Avian keratin disorder, beak deformity, Black-capped Chickadee, serum chemistry, uric acid.

The Black-capped Chickadee (*Poecile atricapillus*) is a small North American passerine that resides year-round in Canada and the northern US (Foote et al. 2010). In Alaska, this species has recently been affected by avian keratin disorder, characterized by gross beak deformities that affect 6.5% of the adult population (Fig. 1; Handel et al. 2010). Although its cause is unknown, underlying histologic changes in the beak and other keratinized tissues are common features (Van Hemert et al. 2013). Associated health effects in affected birds include changes in diet (Van Hemert et al. 2012) and plumage (D'Alba et al. 2011) and an increased occurrence of opportunistic infections (Van Hemert et al. 2013). Such changes may be due to behavioral modifications necessitated by the physical deformity or they may be related to underlying systemic disease.

Serum chemistry parameters are often responsive to acute or chronic diseases in wild birds and can offer insights about an individual's condition. Although reference values have not been established for many species, including Black-capped Chickadees, testing of apparently healthy individuals within a population provides a baseline against which to compare abnormal values that may be of diagnostic utility. In the case of avian keratin disorder, notable deviations from "normal" serum chemistry values could help to identify abnormal physiologic processes that may be associated with the etiology of the deformities.

We tested for differences in serum chemistry parameters between birds with and without beak deformities. Between February and April 1999, we collected blood samples from seven (two male, five female) adult Black-capped Chickadees with severely deformed beaks and 10 (six male, four female) adults with normal beaks for serum chemistry analysis. All birds were captured in south-central Alaska using modified funnel traps or mist nets (Handel et al. 2010). We determined age from plumage characteristics (Pyle 1997) and sex via molecular methods (Handel et al. 2006). We used gross beak measurements, following the criteria of Handel et al. (2010), to identify beak deformities among individuals affected by avian keratin disorder. Birds were held individually in 76×46×46-cm stainless steel cages or aerated cardboard holding boxes at 10 C, with access to ad libitum food (sunflower seed pieces) and water for 1–6 h, except for two individuals (one with a normal beak, one with a deformed beak) which were held for 24 h before processing. We disinfected the skin with 70% isopropyl alcohol and drew blood (50–500 μL) from the jugular vein into a 1-cc syringe with a sterile, 27-ga needle. We immediately euthanized each bird by cervical dislocation. Blood was placed in red-top BD Microtainer® Blood Collection Tubes (Becton, Dickinson and Company, Franklin

FIGURE 1. Black-capped Chickadee (*Poecile atricapillus*) with gross beak deformity characteristic of avian keratin disorder. September 2003, Anchorage, Alaska, USA.

Lakes, New Jersey, USA) and allowed to clot for 30 min before we centrifuged the tubes for 5–10 min at 500 × G. Tubes were air-shipped on ice immediately to Marshfield Laboratories, Marshfield, Wisconsin, USA for protein electrophoresis and testing of blood serum for 17 blood chemistries using the avian profile of tests (Table 1). All work was conducted under appropriate state and federal permits and with approval of the US Geological Survey Institutional Animal Care and Use Committee.

To account for small sample sizes and nonnormal distribution of data, we used the Mann-Whitney *U*-test to compare serum chemistry parameters between birds with and without beak deformities. Because of limited blood volume, not all parameters were measured for each individual, and we excluded parameters with <5 individuals per group from analysis. Limited sample sizes precluded us from including sex as a covariate, although a priori tests of values pooled across beak deformity groups showed that none of the parameters differed between males and females (all $P>0.10$) except for glucose ($P=0.032$). Although glucose levels were lower in males (median 425 mg/dL, range 295–500, $n=7$) than in females (median 505 mg/dL, range 45–775, $n=7$), there was no consistent pattern relative to beak deformity within sex.

Black-capped Chickadees with deformed beaks had significantly lower concentrations of uric acid in their serum than did chickadees with normal beaks ($U=2.50$, $n=13$, $P=0.008$). There were no other differences between groups (all $P>0.10$; Table 1). Normal uric acid values may vary across avian species (e.g., Hill and Murray 1987; Newman et al. 1997; Calabuig et al. 2010) and it is difficult to interpret the biologic significance of this difference without reference values for Black-capped Chickadees. Decreases in uric acid are sometimes associated with fasting or malnutrition (Harr 2006), which is plausible among birds with severe beak deformities and compromised feeding ability, and may reflect the sequelae of chronic avian keratin disorder. Alternatively, elevated levels of uric acid in birds with normal beaks could have resulted from dehydration (Harr 2006), although both groups were held in captivity for similar durations with access to food and water, and we did not observe increases in total protein as would be expected with severe dehydration.

Gamma glutamyl transferase values were elevated in two affected chickadees (15 U/L, 20 U/L), one of which also had unusually low cholesterol (<3 mg/dL) and glucose (45 mg/dL) levels, which could be indicative of liver failure (Harr 2006). Abnormal beak growth is sometimes attributed to liver disease (Lumeij 1994), but we did not detect specific, consistent changes in serum biochemistry that would indicate primary hepatic dysfunction in affected birds (Harr 2002, 2006). This finding concurs with results from necropsies and histologic examination, which similarly did not reveal consistent evidence of liver pathology (Handel et al. 2010; Van Hemert et al. 2013). One bird with a normal beak had high values for glucose (775 mg/dL), aspartate aminotransferase (2295 U/L), alanine aminotransferase (555 U/L), alkaline phosphatase (245 U/L), creatine kinase (20490 U/L), total protein (5 g/dL), and albumin:globulin ratio (1.22) and may have been responding to an undiagnosed inflammatory condition unrelated to avian keratin disorder.

Serum chemistry results did not reveal diagnostic patterns associated with avian keratin disorder. However, avian keratin disorder is characterized by beak deformities that worsen over time, and birds with severe beak deformities are in an advanced, chronic stage of the

TABLE 1. Levels of enzyme activity, concentrations of nutrients, metabolites, and electrolytes, and percentages of different protein fractions in blood serum of adult Black-capped Chickadees (*Poecile atricapillus*) with normal and deformed beaks during winter 1999, Anchorage, Alaska, USA.

Parameter[a]	Normal beaks				Deformed beaks			
	Median	Minimum	Maximum	*n*	Median	Minimum	Maximum	*n*
Enzyme activity								
ALT (U/L)	59.5	4	555	6	95	40	315	6
AP (U/L)	75	3	245	6	55	3	100	7
AST (U/L)	624	435	2,295	7	563	285	1,235	7
CK (U/L)	2,635	1,290	20,490	7	3,030	840	5,385	7
GGT (U/L)	3	3	5	5	4	3	20	6
LDH (U/L)	1,752.5	800	2,320	6	1,957.5	940	2,690	6
Nutrients, metabolites, electrolytes								
Calcium (mg/dL)	6.9	4.7	7.7	5	5.9	2.7	7.6	7
Cholesterol (mg/dL)	214	75	2,085	7	201	1.5	295	7
Glucose (mg/dL)	425	295	775	7	490	45	630	7
Phosphorus (mg/dL)	4.85	4.1	15.1	4	4.25	3.6	4.9	2
Total protein (g/dL)	2.6	1.0	5.0	10	3.2	2.0	3.9	7
Uric acid (mg/dL)	17.75	11.90	42.60	6	8.0	4.5	13.8	7
Chloride (mmol/L)	122	122	122	2	118.5	116	121	2
Potassium (mmol/L)	9.55	8.8	10.3	2	11.05	10.5	11.6	2
Sodium (mmol/L)	158.5	157	160	2	155.5	153	158	2
Bicarbonate (mmol/L)	37	37	37	1	43	42	44	2
Anion gap (mmol/L)	10	10	10	1	5.5	5	6	2
Protein fraction								
Prealbumin (%)	33.94	19.81	34.37	3	28.98	27.23	34.13	4
Albumin (%)	36.06	23.06	41.87	3	36.20	30.34	38.54	4
α1-globulin (%)	5.14	4.64	5.54	3	5.65	4.84	7.75	4
α2-globulin (%)	4.02	3.96	4.10	3	4.73	3.07	6.21	4
β-globulin (%)	6.32	4.10	19.35	3	10.91	5.15	13.95	4
γ-globulin (%)	13.62	11.05	28.53	3	13.70	10.26	18.23	4

[a] ALT = alanine aminotransferase; AP = alkaline phosphatase; AST = aspartate aminotransferase; CK = creatinine kinase; GGT = gamma glutamyl transferase; LDH = lactate dehydrogenase.

disease. It is therefore possible that the inciting factor (whether infectious, toxic, or nutritional) was no longer present at the time of sample collection and thus not reflected in the serum chemistries of the birds we sampled. In addition, definitively classifying individuals affected by avian keratin disorder presents research challenges. The detection of a gross beak deformity is currently the only way to identify avian keratin disorder in live birds, and it is possible that some apparently healthy individuals measured in this study could have been at early stages of the disease, potentially masking differences between groups. Measurement of serum chemistry parameters in a larger number of Black-capped Chickadees from Alaska and other geographic areas where avian keratin disorder is not known to occur would help to elucidate the range of normal values for this species.

Small samples sizes may also have limited our ability to detect differences between groups. It is impossible to control for all sources of variation in wild-caught birds, and both groups had large ranges for most parameters measured. We were unable to include sex, which along with reproductive status can sometimes influence serum chemistry (Harr 2006), as a covariate in our analysis. Although we sampled birds during the nonbreeding season, when hormonal and energetic differences between males and females are likely to

be minimal, we cannot rule out the possible influence of sex as a confounding factor.

Compared to reference values for a range of companion avian species (Harr 2002), chickadees had high levels of aspartate aminotransferase, lactate dehydrogenase, and creatine kinase. These parameters can be influenced by stress associated with capture and handling, are often higher in wild-caught versus captive birds of the same species (Perry et al. 1986; Newman et al. 1997; Naidoo et al. 2008), and may increase with holding time (Franson et al. 2009). Although Black-capped Chickadees typically tolerate captive conditions well (Foote et al. 2010), relatively long holding times prior to blood sampling may have affected aspartate aminotransferase, lactate dehydrogenase, and creatine kinase measurements. However, these and all other serum chemistry parameters from our study were within the range of values reported from other apparently healthy wild-caught birds (e.g., Newman et al. 1997; Naidoo et al. 2008; Calabuig et al. 2010) and some captive passerines (Hill and Murray 1987). Additional work is required to determine appropriate reference values for Black-capped Chickadees in the wild.

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