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Serum Biochemistry and Electrophoretic Patterns in the Eurasian Buzzard (*Buteo buteo*): Reference Values

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ABSTRACT: In avian medicine, hematologic and biochemical laboratory investigations are still in their infancy, because of the difficulty involved in collecting data. This has led to a lack of reference values and a nonstandardized approach to specimens obtained in critical conditions. The Eurasian Buzzard (*Buteo buteo*) is one of the most common raptors in Italy, yet little is known about the physiologic blood parameters of this species. Serum biochemistry and electrophoretic investigations were performed in 40 healthy Eurasian Buzzards in different Italian wildlife rescue centers waiting to be released after recovering from trauma injuries. Mean values for biochemistry parameters were pancreatic amylase 626.9 IU/l, uric acid 7.5 mg/dl, aspartate aminotransferase 330.9 IU/l, glucose 375.1 mg/dl, lipase 26.3 IU/l, total protein 38.4 g/l, total bilirubin 0.04 mg/dl, lactate dehydrogenase 2,008.4 IU/l, creatinine kinase 1,604.1 IU/l, alanine aminotransferase 40.4 IU/l, alkaline phosphatase 89.8 IU/l, magnesium 2.3 mg/dl, calcium 10.2 mg/dl, phosphorus 2.02 mg/dl, cholesterol 192.2 mg/dl, triglyceride 116.4 mg/dl, albumin 14.5 g/l, creatinine 0.1 mg/dl. Mean electrophoretic values were prealbumin 1.4 g/l, albumin 14.2 g/l, alpha 1 globulin 5.9 g/l, alpha 2 globulin 4.7 g/l, beta globulin 7.5 g/l, gamma globulin 3.6 g/l, albumin/globulins ratio 0.8 g/l.

Key words: Bird of prey, blood biochemistry, *Buteo buteo*, electrophoresis, fraction, protein, raptor.

The Eurasian Buzzard (*Buteo buteo*) is one of the most common species of raptors in Italy (Clark, 1999) where it utilizes many different habitats from open ground to small woods and forests. Despite the ease with which it adapts to different environmental conditions and cohabits with humans, anthropogenic factors (e.g., poisoning, hunting, and habitat invasion) have had an important impact on the biologic and health population status of this raptor, and it is now one of the most common species submitted for rehabilitation in wildlife centers in Italy. Although some hematologic and biochemistry parameters for this bird of prey are reported in literature (Hernandez, 1990), others are not available. There is a similar lack of published data on the electrophoretic patterns of serum or plasma protein for *B. buteo*. The objective of the present study was to investigate the serum biochemistry and electrophoretic values in 40 healthy wild Eurasian Buzzards to provide mean values that are not currently available in the literature.

From June to July 2004, blood samples were collected from 40 prerelease Eurasian adult buzzards (22 females and 18 males) following medical and surgical care at Wildlife Centers (Treviso and Belluno, Italy), at a private veterinary clinic (Clinica Einaudi, Bari, Italy), and at the Faculty of Veterinary Medicine of the University of Padua (Italy). Animals were selected on the basis of gonadal inactivity, ascertained by means of an endoscopic evaluation. The rehabilitation period prior to onset the study ranged from 3 to 8 mo, depending on severity of disease or trauma occurring in each case. During the rehabilitation period all birds were given the same diet (dead birds and rodents). By analyzing clinical records with regard to radiographic, endoscopic, and clinical

chemistry reports (data not shown) 40 healthy buzzards were selected. While birds were awake and manually restrained, blood samples (1 ml) were drawn from the brachial vein with 2.5-ml syringes (26-gauge needle) for biochemistry and electrophoresis. Blood was placed in 6-ml vacutainer tubes without an anticoagulant, was kept at 22–25 C for 20 min to allow clotting, and then centrifuged for 10 min at 700 × G. Serum samples were analyzed within 1 hr of blood collection. Serum was used for electrophoresis, as fibrinogen in plasma can obscure the electrophoretogram in the β–γ region (Thomas, 2000).

Serum biochemistry pattern was determined for each sample with the use of a Hitachi 912 Automatic Analyzer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany) following the assay method validated and warranted by Roche Diagnostics. The following biochemistry analyses were performed on serum samples: glucose (GLU), pancreatic amylase (P-AMY), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (AP), lipase (LIP), creatinine kinase (CK), lactate dehydrogenase (LDH), cholesterol (CHOL), triglyceride (TG), calcium (Ca), phosphorus (PHOS), magnesium (Mg), total bilirubin (T-BIL), urea (UREA), uric acid (UA), creatinine (CREAT), total protein (TP), and albumin (ALB). Serum protein separation was achieved with the use of the P/ACE MDQ Capillary Electrophoresis System (Beckman Coulter, Inc., Fullerton, California, USA) equipped with automatic constant-volume sample injection devices and capillary temperature control. System control, data collection, and analyses were made with the respective software for each piece of equipment. Untreated fused-silica capillaries of 50-cm length and 50 μm (inside diameter) were used. The running buffer was 60 mM sodium tetraborate pH 9.4 (SIGMA Chemical Co., St. Louis, Missouri, USA) and the capillary temperature during the analysis was 20 C. Diluted samples (5 μl serum with 120 μl water) were introduced by pressure injection (5 or 10 sec at 0.8 psi) followed by protein separation at a constant voltage of 25 kV. The total run time was 10 min and the serum proteins were detected on-line at 200 nm. Between runs, the capillary was rinsed for 1 min with 1 N NaOH, 0.5 min with water, and 1 min with running buffer. The protein fractions performed and investigated were prealbumin, albumin, alpha, and beta and gamma globulins.

Data obtained were analyzed with a nonparametric method for estimation of mean of reference values, standard deviation (SD), standard error (SE), and 95% confidence limits (CL) (Solberg, 1993; Fowler and Cohen, 2002).

Values obtained for the biochemistry parameters found in the animals used are reported in Table 1. Values for serum proteins are reported in Table 2. A standard electrophoretogram of *B. buteo* is shown in Figure 1.

The aim of the present study was to characterize and establish normal reference ranges for both biochemistry values and electrophoretographic patterns in the Eurasian Buzzard. Several blood chemistry values such as glucose, cholesterol, aspartate–amino transferase obtained in this study are similar to those reported for other *Buteo* species (Hernandez, 1990; Samour and D'Aloia, 1996; Samour, 2000). Concentration of some enzymes (such as creatine kinase and lactate dehydrogenase), appeared to be very high in these species, despite the good physical condition of evaluated birds. The cause of such increased values might be that both these enzymes are sensitive to stress and may rise as a result of contamination from tissue fluid during sampling. In our series, blood samples were collected a few minutes after animals were immobilized in aviaries in order to minimize any stress-related increase in serum glucose levels; in fact, the levels recorded for this metabolite appeared to be normal (Hernandez, 1990). Alkaline phosphatase levels ap-

TABLE 1. Biochemistry parameters in *Buteo buteo*.

Parameters[a]	Mean	SD	SE	95% confidence limits	Range (minimum–maximum)
P-AMY (IU/l)	626.930	414.193	130.979	626.93±296.28	329.5–1689.4
UA (mg/dl)	7.534	5.429	1.717	7.534±4.498	3.04–20.22
ASAT (IU/l)	330.900	72.478	22.919	330.9±60.047	2.38–4.80
GLU (mg/dl)	357.100	45.217	14.299	357.100±37.463	299–401
LIP (IU/l)	26.370	5.144	1.627	26.370±4.263	26.0–39.4
UREA (mg/dl)	12.340	3.268	1.034	12.30±2.709	8.7–18.0
TP (g/l)	38.450	5.909	1.869	38.45±4.90	30.5–47.8
T-BIL (mg/dl)	0.040	0.023	0.007	0.040±0.018	0.02–0.07
LDH (IU/l)	2,008.400	314.614	99.490	2,008.4±260.664	1,383–2,261
CK (IU/l)	1,604.100	649.109	205.266	1,604.1±537.8	960–,2672
ALAT (IU/l)	40.400	8.909	2.817	40.400±7.380	32–59
ALP (IU/l)	89.800	27.764	8.780	89.800±23.004	59–149
Mg (mg/dl)	2.319	0.337	0.107	2.319±0.280	1.82–2.91
CA (mg/dl)	10.238	0.874	0.276	10.238±0.723	9.65–12.01
PHOS (mg/dl)	2.020	1.438	0.455	2.020±1.192	0.7–5.6
CHOL (mg/dl)	192.200	28.836	9.119	192.200±23.892	136–244
TG (mg/dl)	116.400	52.502	16.603	116.400±43.500	56–216
ALB (g/l)	14.590	1.081	0.342	14.590±0.896	13.0–16.2
CREAT (mg/dl)	0.154	0.091	0.029	0.154±0.076	0.06–0.33

[a] P-AMY = pancreaticamylase, UA = uric acid, ASAT = aspartate–aminotransferase, GLU = glucose, LIP = lipase, UREA = urea, TP = total protein, T-BIL = total bilirubin, LDH = lactate dehydrogenase, CK = creatinine kinase, ALAT = alanine aminotransferase, ALP = alkaline phosphatase, Mg = magnesium, CA = calcium, PHOS = phosphorus, CHOL = cholesterol, TG = triglyceride, ALB = albumin, CREAT = creatinine.

peared to be lower than those reported in many other species of birds; however, the great variation in the analysis methods used by different authors precludes a reliable determination of the baseline level of this enzyme. The values obtained in the present study for metabolites such as creatinine and uric acid appeared to be

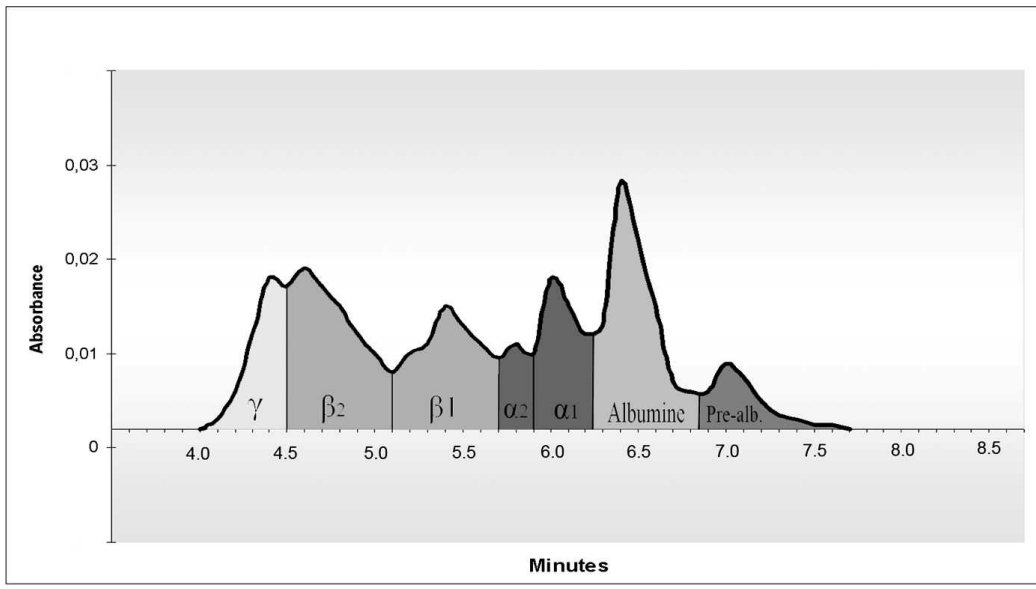

FIGURE 1. Standard electrophoretic pattern of an adult Eurasian Buzzard (*Buteo buteo*). Different times of migration of different protein fractions (depending on their electric charge shape and size) can be easily appreciated.

TABLE 2. Serum protein fractions.

Parameters[a]	Mean	SD	SE	95% confidence limits	Range (minimum–maximum)
PRE-ALB (g/l)	1.469	1.751	0.554	1.469±1.441	0.92–3.37
ALB (g/l)	15.230	2.545	0.805	15.230±2.109	11.84–19.25
α 1 (g/l)	5.939	1.258	0.398	5.939±2.460	4.31–8.67
α 2 (g/l)	4.742	2.360	0.746	4.742±1.954	2.72–9.28
β (g/l)	7.525	1.607	0.508	7.525±1.331	4.81–10.12
γ (g/l)	3.685	1.7505	0.5536	3.685±1.451	1.54–6.01
A/G (g/l)	0.8023	0.1887	0.0597	0.802±0.157	0.498–1.038

[a] PRE-ALB = prealbumin, ALB = albumin, (α 1) = alpha 1 globulin, (α 2) = alpha 2 globulin, β = beta-globulin, γ = gamma-globulin, and A/G = albumin–globulins ratio.

much lower than those reported by other authors for the European Buzzard (Hernandez, 1990), but within the ranges reported for avian patients (Hochleithner, 1994). No reference value for triglycerides for *B. buteo* are available; this metabolite has also been reported to increase in the avian patient after exercise and exposure to stress (Hochleithner, 1994). Further studies are therefore required to ascertain whether our findings may have been influenced by the physical manipulation of the raptors. To our knowledge, no reference ranges for amylase and lipase serum concentration in this species are available, although the values for amylase found in our subjects may be considered within the ranges reported for healthy avian patients (Hochleithner, 1994; Samour, 2000).

In mammalian medicine, electrophoretographic patterns providing information on abnormal levels of proteins or single fractions (along with other diagnostic methods) may be helpful in orienting clinicians in the diagnosis of some specific diseases (e.g., a monoclonal peak in the beta fraction may occur in patients with multiple myeloma or lymphoma). Also, in the acute phase of some infections, changes in serum proteins are detected early, even when serologic tests fail to detect the infectious agent (Thomas, 2000). In birds, changes in total blood protein in some fractions, such as albumin and gamma globulins, are a useful diagnostic tool in the evaluation of physical condition (starvation, dehydration, inflammatory processes), providing information for diagnosis, monitoring, and prognosis (Cray et al., 1996; Cray and Tatum, 1998; Cray, 2000; Tatum, 2000; Lanzarot, 2001; Blanco and Hofle, 2003; Roman et al., 2005) as well as information on pathologic conditions (Lumeij, 1997).

In birds and reptiles, changes in plasma proteins are not exclusively related to disease, but may also be physiologic, being correlated to age, sex, season, and reproductive status (Kurye and Gasparaska, 1985; Quesenberry, 1991; Rosenthal et al., 2005). Although electrophoresis is gaining importance as a diagnostic tool in avian medicine (Tatum, 2000; Gelli et al., 2005, 2006; Ordonneau et al., 2005), physiologic and pathologic patterns of electrophoretograms in different species must be established for it to be useful. In the buzzards in our study (Fig. 1), a low to negligible prealbumin fraction was present, migrating within a time interval of 7–7.5 min; prealbumin appears in the farthest area of migration. Albumin was represented by a high peak shouldered by the α fraction. As in other birds and mammals (Lumeij, 1997; Thomas, 2000), globulins consist of a heterogeneous group of proteins with a weaker negative charge that migrate at a slower rate than albumin, migrating in *B. buteo* within a time interval of 4–6.5 min. Values for beta globulins in our patients were slightly lower than those reported in literature for other species of raptors (Tatum, 2000;

Blanco and Hofle, 2003). This is because we used serum instead of plasma to evaluate β globulins in healthy animals without fibrinogen, which as an acute-phase reactant of this fraction, may be a confounding factor, masking the true value of this globulin. Gamma and beta globulins appear in a bridged peak in the electrophoretograms, not as distinct fractions.

The scarcity of reference values on hematology and serology in wild animals is probably due to difficulty involved in collecting useful data from free-ranging specimens and, in some cases, the fact that wild bird medicine is of little commercial interest. It is therefore often impossible to perform routine laboratory tests, especially in a wildlife rehabilitation center. The lack of standard values hinders the use of both biochemistry and electrophoresis in many species of wild birds, which creates a vicious circle when one attempts to obtain diagnostic information. This is the first study reporting values of electrophoretic patterns of protein fraction in serum of the European Buzzard, although further studies are needed to improve our present knowledge, the values obtained in our study may be considered as reference for protein electrophoresis in this species.

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