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Establishment of selected baseline values of blood chemistry of Himalayan Griffon *Gyps himalayensis*

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Abstract
The study attempted to obtain baseline information for plasma metabolites and blood enzymes of Himalayan Griffon *Gyps himalayensis*. The Himalayan griffons sampled in the study were rescued by the Assam Forest Department and the Bombay Natural History Society, from various mishaps. 3 ml of whole blood was collected from each vulture and biochemical analysis was conducted. The levels of Aspertate Amino transferase (AST), Creatine Kinase (CK) uric acid, bile acid, protein, albumin, globulin, A:G, glucose, calcium, phosphorus, sodium and potassium were estimated in Vetscan. The total cholesterol, triglyceride, creatinine, bilirubin, LDH, ALT, ALP and chloride were estimated spectrophotometrically using commercially available kits. The hormones T_3, T_4 and cortisol were estimated by radio immune assay (RIA) I-125 gamma counter. Although the data set is small it would be valuable for establishing the baseline information on blood bio-chemistry of Himalayan Griffon.

Keywords: Himalayan Griffon *Gyps himalayensis,* vulture**,** blood biochemistry, baseline data spectrophotometer, RIA I-125 gamma counter

1. Introduction
Himalayan Griffon *Gyps himalayensis* is a huge bird and is the largest and heaviest of all vultures found in India. This species is found along the Himalayas and in Central Asia. Vultures are scavengers and they are most efficient, nature's own disposal squads. Since 1990s the populations of the 3 resident species of *Gyps* vultures in India have declined by more than 99% and now they are considered to be critically endangered birds [1, 2]. The main cause of crashes in resident *Gyps* vulture population was found to be the use of the drug diclofenac, a Non Steroidal Anti Inflammatory Drug (NSAID) in treating livestock [3, 4].The vultures get exposed to the drug when they feed on the carcass of an animal which died within 72 hours of administration of the drug. The drug is extremely toxic to vulture and they die of kidney failure [5].

Several national and international conservation organizations are working to save the vultures from extinction. The Bombay Natural History Society in collaboration with Assam Forest Department established the third Vulture Conservation Breeding Centre of the country in 2007 at Rani, Assam. The centre was established for Oriental White-backed Vulture G. *bengalensis* and Slender-billed vultures *G. tenuirostris*. The centre, apart from its conservation breeding programme of the resident *Gyps* vultures, also rescued and treated injured Himalayan griffons following natural calamities, accidents and unintentional poisoning in collaboration of Forest Department of Assam.

The objective of this study was to establish the baseline values of blood metabolites of Himalayan Griffon *G. himalayensis* as very little information on the blood chemistry of this species is available. Haematological and blood chemistry values can be obtained easily and are useful in determining the general health condition of birds [6]. The complete blood count (CBC) enables rapid diagnosis and treatment. The CBC is probably the most useful of all diagnostic tests. To rehabilitate individual raptors or to breed and release endangered birds-of-prey, information of their normal concentrations of blood constituents is of paramount importance. Understanding of the causes of death, illness, and injury can improve and facilitate the application of management measures for the conservation of endangered species [7]. To provide right veterinary care for this endangered species, veterinarians and rehabilitators need accurate hematological and biochemical reference ranges on the basis of which veterinary evaluations and treatment could be initiated.

Clinical hematology and blood chemistry are useful diagnostic tools in clinical practice [8] and are especially important in birds, which frequently show few overt clinical signs of disease. Haematology and blood chemistry have also been shown to be useful tools for evaluating the general health of birds in wild and captive populations and for the rehabilitation of injured individuals [9]. Although several studies have been published in recent years on the normal hematology and blood biochemistry values on various species, little information on the blood chemistry of Himalayan Griffon *G. himalayensis* is available. Thus a study was designed to establish the baseline values of some selected biochemical metabolites of Himalayan Griffon *G. himalayensis*.

2. Methods and Materials

2.1 Ethical approval

The prior approval from the Institutional Animal Ethical Committee was obtained for collection of blood from Himalayan Griffon *G. himalayensis* vulture for the present study.

2.2 Experimental design

Fifteen Himalayan griffons sampled during the study were rescued between the years 2011 and 2014 from different accidents by Assam Forest Department and Vulture Conservation Breeding Centre, Rani, Assam. These birds were subsequently released in the wild after they recovered. All rescued vultures were housed at temporary Quarantine Aviaries at Nalapara which is situated at 2 km from Vulture Centre. The aviaries were designed especially for vultures and were of dimensions 20'x20'x12'. The aviaries were made of iron poles and chain-link iron fences on all sides. Aviaries were open to sky with netting of iron wire mesh on top. An extra layer of nelton was provided from inner side to protect from possible injuries to the vultures. Wooden perches wound with coir ropes and water troughs were provided within each aviary. All birds were juvenile or sub-adult and were between 1 to 3 years of age. All the sampled birds appeared healthy on visual examination. Sex identification could not be made because the birds are not sexually dimorphic. However the plasma values of blood do not differ much between sexes as was shown by the study done on Egyptian Vultures [10]. The captive vultures were fed on freshly slaughtered goat carcasses.

2.3 Biochemical assay

Blood samples were collected from birds aseptically during early morning by venipuncture of the medial metatarsal vein using a 23 guage hypodermic needle with 3ml syringes. Whole blood was collected into 5ml vacuumtainer tube containing EDTA as the anti-coagulant. Up to 3 ml of blood per bird was collected. The cellular portion of the blood was removed by centrifugation at 3000 rpm for 5 minutes and the plasma was immediately frozen at - 2°C. Biochemical analyses were subsequently conducted. All analyses were done in duplicate to overcome possible errors. The amount of AST, CK, uric acid, bile acid, protein, albumin, globulin, A:G, glucose, calcium, phosphorus, sodium and potassium were estimated in Vetscan, VS 2 Abaxis. The total cholesterol, triglyceride, creatinine, bilirubin, LDH, ALT, ALP and chloride were estimated spectrophotometrically using Systronic 20 using commercially available kits [11]. After processing the samples and standards provided with the kits, absorbance of the standard and the samples was determined. Concentration of the biochemical constituents was calculated according to the manufacturer's instruction.

2.4 Hormonal assay

The hormones tri-iodothyronine (T_3), tetra-iodothyronine (T_4) and cortisol concentration in plasma were estimated by radio immune assay (RIA) I-125 gamma counter [12].

3. Results

The mean ± S.E. of blood metabolites, enzyme activities and hormone concentration are shown in table 1, 2 and 3. The total protein, albumin, globulin concentration were recorded to be 4.601±0.316, 2.580±0.341, 2.021± 0.218 g/dl respectively, and A:G ratio was 1.529±0.341. The mean blood glucose, cholesterol, triglyceride, creatinine, total bilirubin, uric acid level were found to be 253.666±12.026, 156.486±4.752, 77.656±3.656,0.248±0.038, 0.135±0.026, 3.683±0.238 mg/dl. The concentration of calcium, phosphorus were 9.220±0.399 and 3.927±0.39 mg/dl respectively while that of sodium, potassium and chloride ion and bile acid were 149.458±6.973, 2.797±0.295, 84.552±3.613 and 35±0.00µmol/L respectively.

Table 1: Mean ± S.E. of blood metabolites of Himalayan Griffon *Gyps himalayensis*

Blood metabolites	Concentration	Minimum concentration	Maximum concentration	Concentration of blood metabolites reported by earlier workers [13] in Eurasian Griffon	
				Mean	Range
Total Protein (g/dl)	4.601±0.31	3.09	6.876	3.9	3.0-5.1
Albumin (g/dl)	2.580±0.34	1.851	4.987	1.77	1.3-2.23
Globulin (g/dl)	2.021± 0.21	1.006	3	0.76	0.52-2.16
A:G ratio	1.529±0.34	0.662	3.957	2.32	No report
Glucose (mg/dl)	253.6±12.02	186.6	309.09	291.89	255.86-400.00
Cholesterol (mg/dl)	156.486±4.752	135.656	176.912	197.23	119.88-274.56
Triglyceride (mg/dl)	77.656±3.656	61.042	96.930	97.43	49.59-168.28
Uric acid(mg/dl)	3.683±0.238	2.098	4.870	5.51	2.81-11.39
Total bilirubin (mg/dl)	0.135±0.026	0.011	0.276	No report	No report
Creatinine(mg/dl)	0.248±0.038	0.123	0.540	0.74	0.35-0.98
Calcium(mg/dl)	9.220±0.399	7.043	10.985	14.00	9.60-18.80
Phosphorus(mg/dl)	3.927±0.39	2.093	5.987	13.31	8.67-19.81
Sodium(µmol/L)	149.458±6.973	117.090	187.340	154.90±6.70	144-197
Potassium(µmol/L)	2.797±0.295	1.908	4.764	2.100±0.90	1.800-3.400
Chloride(µmol/L)	84.552±3.613	65.098	96.097	98.200±12.2	85.50-114.300
Bile acid (µmol/L)	35±0.00	35.0	35.0	No report	No report

Table 2: Mean ± S.E. of enzyme activities of Himalayan Griffon *G. himalayensis*

Enzyme	Enzyme activities	Minimum conc.	Maximum conc.	Range of Enzyme activities reported by earlier workers [13] in Eurasian Griffon
AST(IU/L)	333.741±32.244	234.987	401.121	93.5 -156.8
ALT(IU/L)	29.252±3.087	12.876	43.765	3.9-19.8
ALP(IU/L)	45.465±4.000	23.765	54.987	30.0- 354.4
CK(IU/L)	389.336±51.336	147.980	603.876	103.0-774.0
LDH(IU/L)	433.666±47.234	213.870	623.987	103.0-586.0

The enzyme activities of AST, ALT, ALP, CK and LDH shown in table 2 were observed to be 333.741±32.244, 29.252±3.087, 45.465±4.000, 389.336±51.336 and 433.666±47.234 U/L respectively.

Table 3: Mean ± S.E. of hormonal parameters of Himalayan Griffon *G. himalayensis*

Hormones	Concentration	Minimum conc.	Maximum conc.	Concentration reported by earlier workers on *Gyps* vultures
T_3(mmole/L)	1.214	0.11	2.33	No earlier report available
T_4(mmole/L)	0.680	0.217	0.924	
Cortisol (mmole/L)	76.648	2.33	54.33	

The serum cortisol, T_3 and T_4 concentration shown in table 3 were observed to be 76.648, 1.214 and 0.680 mmole/L respectively.

4. Discussion

There is no previous information on the blood biochemical parameters of Himalayan Griffon. However studies have been done on closely related Griffon Vulture *G. fulvus* kept in captivity in Barcelona Zoo and the blood values were compared with the recorded values on the closely related Griffon Vulture [12]. All birds were adults, between 4 and 20 years old, with no clinical signs of diseases. The present study showed that the range of enzyme activities were familiar similar to that reported of Eurasian Griffon Vulture except for AST and ALT. All the blood metabolites were within the range of Eurasian Griffon published information [13] except for phosphorus and creatinine. The concentration of these parameters probably differed from that of Griffon vulture's study [14] could be due to the difference in conditions in captivity, such as diet, physical activity and species of the vulture. The enzymes AST, ALT and ALP and protein, albumin and bilirubin are important liver biomarkers and their baseline values are important to know if the bird suffers from any liver dysfunction. Bile acids have been found to be the most sensitive indicator of liver disease in birds [15]. Their concentration indicates the clearing capacity of the liver. Measurement of serum CK is a useful aid in distinguishing between muscle and liver disease in birds. Since CK is found primarily in cardiac and skeletal muscle, and usually indicates a muscular condition. Elevations occur with damage to skeletal muscle (e.g., from injections, trauma, and feather picking) and with myocardial (heart muscle) disease [15]. The LDH isozyme studies in muscles of birds are important as they might be helpful in unraveling the metabolic and neurotrophic phenomena occurring in atrophic muscle disorders [14]. Elevated values of LDH are most common with liver disease in birds. Elevations may also occur with heart or muscle damage. Low protein levels may indicate malnutrition, malabsorption, chronic disease, renal disease, liver disease, parasitism, or stress. Elevated values indicate dehydration, shock, or infection [15]. Hypoglycemia (low blood sugar) occurs with malnutrition, liver disease, fasting, and systemic disease. Hyperglycemia (high blood sugar) may occur during breeding, stress, egg yolk peritonitis and diabetes mellitus while elevated levels can be seen in birds on high-fat diets, obese birds, and birds with hypothyroidism (underactive thyroid gland). Low levels can be seen in birds with liver and kidney disease. Uric acid and creatinine are the primary nitrogenous waste product of the avian kidney, and its level in the serum is an excellent indicator of renal function or kidney biomarkers.

The primary role of electrolytes lies in the maintenance of body ionic and water balance. The blood electrolyte balance (EB) is also closely associated with blood pH, gas pressure and HCO_3 concentration [16]. The concentration and balance of electrolytes in a bird's intracellular and extracellular fluids are critical for life and are therefore tightly regulated. Prevention of electrolyte imbalance should be obviously approached through incorporation of appropriate cations and anions in diet formulations [17]. Low calcium levels, which are frequently seen as the cause of seizures in birds, can result from poor calcium supplementation in the diet, renal disease, and other metabolic conditions. Ovulating birds have elevated calcium levels, apparently related to the calcium needed for eggshell formation [16].

No previous reports are available regarding the hormones T_3, T_4 and cortisol of *Gyps* vultures. The thyroid gland is involved in the regulation of growth, development, adaptation and productivity. The level of thyroid hormone synthesis regulates the rate and the direction of metabolic events, determines their physiological optimum [9]. The hormone cortisol is a stress hormone. This discussion will hopefully give a better insight into the "mysteries" of vulture blood profile. By understanding these tests it will assist us to give a better diagnosis and hence a better treatment.

5. Conclusion

The study has attempted to determine normal values for plasma metabolites, enzyme activities and some hormones of Himalayan Griffon. As no previous data is available on Himalayan Griffon *G. himalayensis* so these baseline values will be of immense help to the researchers as well as to the veterinarians. These data are of ecological, metabolic, taxonomic, and veterinary interest. Although the data is small it would be valuable for establishing the baseline data of blood biochemistry of Himalayan Griffon. In future, these experimental findings in Himalayan griffons can be extrapolated in the critically endangered Gyps vultures for better veterinary care. However, further studies of hematologic and blood chemistry responses to disease are still needed as a tool in the assessment of pathologic conditions.

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7. Competing interests

The authors declare that they have no competing interests.

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