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Blood biochemistry of White Storks (Ciconia ciconia) across Bulgaria: baseline data for conservation and rehabilitation

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Abstract. We obtained 175 blood samples from wild White Storks in Bulgaria, and determined the values of 18 indicators - alanine transaminase, albumin, alkaline phosphatase, amylase, aspartate transaminase, calcium, chloride, cholesterol, creatine kinase, creatinine, blood glucose, lactate dehydrogenase, magnesium, phosphorus, total bilirubin, total protein, triglycerides and uric acid. In this research, we compared those results with regards to the location from which the birds came from - we conditionally separated Bulgaria into four geographical regions, and to their age. We found statistically significant differences in two parameters - albumin, between northwestern and northeastern populations, and creatinine, between northeastern and southeastern populations. There was also a statistically significant difference in the albumin values between juvenile and adult storks, which we found to be decreasing with age.

Key words: *Ciconia ciconia*, White Stork, blood biochemistry, veterinary medicine.

Introduction

The White Stork (*Ciconia ciconia*, Linnaeus, 1758) is a large migratory bird in the stork family Ciconiidae, with an increasing world population (BirdLife International, 2016). In Bulgaria it is breeding, passing through, and on exception – wintering (Petrov et al., 2015). It is listed as a vulnerable species in the Bulgarian Red Data Book (Petrov et al., 2015), however, the population in the country has increased by 21% from 2004-2005 (4818 breeding pairs) to 2014-2015 (5825 breeding pairs) according to Cheshmedzhiev et al. (2016).

White Storks inhabit various open habitats, including grasslands, wetlands, agricultural fields, and urban areas. They often build their large nests made of sticks on tall structures such as trees, cliffs, or man-made structures. In Bulgaria, their nests are most commonly found on electricity pylons, buildings and chimneys in the rural areas all over the country. In the village of Belozem, part of the European Stork Villages Network, there is the largest gathering of White Stork nests on a single building in the country - over 20 nests (Gradev et al., 2023).

The diet of the storks mostly includes various invertebrates, fish, amphibians, snakes, lizards, and small mammals, alongside fragments or objects stemming from anthropogenic activities (Bjedov et al., 2024; Mikula et al., 2024).

Due to differences in environmental conditions like altitude, food variability, and proximity to water sources and pollutants, individual wild populations even from a small country potentially can have contrasting biochemical components in their blood test results.

Prior studies on White Stork biochemistry show some parameters vary between age groups (Montesinos et al., 1997) and between birds of different gender (of the same age group) (Jerzak et al., 2010). Similar age-related differences have been observed between free-living Black Storks (*Ciconia nigra*) (Lanzarot et al., 2015). This has been reported for other bird species as well - like vultures (Petrov et al., 2023b) for example. The values of Ravens (*Corvus corax*) in Bulgaria on the other hand, have been found to differ between regions (Chaprazov et al., 2023), and those of Short-toed Eagles (*Circaetus gallicus*) in Spain differed between captive and wild individuals (Baumbusch et al., 2021). There are prior studies evaluating some blood plasma parameters of free-living White Stork chicks (Puerta et al., 1989), free-living White Stork adult birds (Alonso et al., 1991), 129 juveniles - both wild and captive-bred White Storks in Spain (Montesinos et al., 1997), 80 captive adults from Hungarian zoos (Szabó et al., 2010), and of 342 chicks from nests in Poland (Jerzak et al., 2010).

A total of 2963 White Storks were accepted in the Wildlife Rehabilitation and Breeding Centre (WRBC), part of Green Balkans - Stara Zagora NGO between 1995-2019 (Petrov et al., 2023a). In this study we established 18 biochemical values by sampling 175 wild White Storks admitted to the WRBC, during 2022 and 2023. We assessed the effect of habitat and of age in order to gain more in-depth knowledge and perform better clinical work regarding this numerous species in Bulgaria.

Materials and methods

This research was carried out on wild White Storks admitted during the period 2022-2023 to the WRBC for treatment, rehabilitation, rearing or fostering. All birds were examined by a veterinary physician and their health status was assessed. After primary examination, 1.5 ml blood from either the left or right cutaneous ulnar vein or either left or right medial dorsal metatarsal vein was collected. We used 3 ml heparin tubes with 23G needles. Samples were processed within 4 hr of collection using a BS-120 (Mindray, China) automatic biochemical analyser. The results were separated into four groups based on the regions in which the birds were found (North-west, North-east, South-west, and South-east Bulgaria), and into three age groups. The age categories were as follows - juv-0 were individuals in their first calendar year, juveniles were individuals from their second calendar year up to reaching breeding age, and adults were breeding storks in adult plumage.

We tested for 18 biochemical parameters - alanine transaminase (ALT, U/I), albumin (g/l), alkaline phosphatase (ALP, U/I), amylase (U/I), aspartate transaminase (AST, U/I), calcium (mmol/l), chloride (mmol/l), cholesterol (mmol/l), creatine kinase (CK, U/I), creatinine (µmol/l), blood glucose (mmol/l), lactate dehydrogenase (LDH, U/I), magnesium (mmol/l), phosphorus (mmol/l), total bilirubin (µmol/l), total protein (g/l), triglycerides (TG, mmol/l), and uric acid (µmol/l).

To assess overall differences in measured blood parameters among the four studied regions as well as among the three age categories across the study period, the one-way analysis of variance (ANOVA) was performed, using measured values as response variable and both regions and age as explanatory variables, respectively. After confirming any statistical significance in the ANOVA, a post hoc Tukey's multi comparison test to assess regional/age differences for each blood parameter was performed. If there were any statistical significance, lowercase letters above mean values (i.e. a, b) were used for indicating regional/age comparisons. The same lowercase letters in each blood parameter indicate significant differences revealed by the Tukey's multi comparison test, whereas the different letters - non-significant. All statistical analyses were performed using IBM SPSS Statistics 26.

Results

All 175 White Storks were examined by a wildlife veterinary specialist prior to the blood collection, and were determined to be clinically normal. The mean, minimum and maximum values of 18 biochemical parameters of all sampled storks divided by four geographic regions in Bulgaria are presented in Table 1. The mean, minimum and maximum values of 18 biochemical parameters of all sampled storks separated by age are shown in Table 2. The compiled data in Table 1 shows significant differences in two parameters - albumin (between northwestern and northeastern populations) and creatinine (between northeastern and southeastern), and there are also noticeable differences in the AST and ALT values between north and south populations. Table 2 shows there was a statistically significant difference in the albumin values between juvenile and adult storks. The LDH value of juv-0 storks is visibly lower than that of juveniles and adults.

Some of the values have visibly wide ranges (e.g. AST, ALP, LDH, TG and etc.). AST can be elevated in liver disease but is found in other tissues, including skeletal muscle, so is a non-specific indicator for this type of disease (Harr, 2006; Haynes & Hollwarth, 2022). Unlike mammals, in birds ALT and ALP are not considered useful in detecting liver damage, so wide variations in these parameters are not alarming. ALT in birds is very non-specific for the liver, and normal levels have been shown in cases with severe liver damage (Doneley, 2007).

Many studies of serum biochemical parameters show wide ranges in serum amylase in healthy individuals. Among mammals, wide amylase ranges are common in humans (Muneyuki et al., 2012), rats (Gulalp et al., 2007) and others, while in birds such values are detected in imperial eagles (Petrov et al., 2024) and some other species. Among companion birds, differentiating levels of amylase are observed in cockatiels and cockatoos (Harr, 2002).

Table 1. Mean, minimum and maximum values of White Storks' blood parameters in four regions in Bulgaria. The same lowercase letters in each blood parameter indicate significant differences revealed by the Tukey's multi comparison test, whereas the different letters - non-significant.

Region	North-west Bulgaria		North-east Bulgaria		South-west Bulgaria		South-east Bulgaria	
Parameter	**n**	**$\bar{X}$ (min-max)**	**n**	**$\bar{X}$ (min-max)**	**n**	**$\bar{X}$ (min-max)**	**n**	**$\bar{X}$ (min-max)**
AST U/l	37	403.19 (119.00-1000.00)	38	374,76 (52.00-800.00)	54	353.07 (155.00-632.00)	46	368.09 (100.00-553.00)
ALT U/l	37	114.73 (48.0-281.0)	38	107.63 (41.00-536.00)	54	90.76 (20.00-287.00)	46	86.54 (47.00-141.00)
ALP U/l	37	983.22 (70.00-3258.00)	38	894.18 (142.00-2387.00)	54	990.57 (132.00-2879.00)	46	955.24 (85.00-2087.00)
LDH U/l	37	1208.19 (474.00-2175.00)	38	1223.11 (210.00-2709.00)	54	1234.20 (607.00-3028.00)	46	1172.04 (369.00-2693.00)
CK U/l	37	1184.38 (245.00-3064.00)	38	1193.32 (236.00-2827.00)	54	1194.22 (303.00-2745.00)	46	1054.13 (359.00-2552.00)
Cholesterol mmol/l	37	6.71 (1.79-11.23)	38	6.71 (4.33-13.30)	54	6.54 (2.31-10.80)	46	6.71 (4.39-10.89)
TG mmol/l	37	0.80 (0.23-1.28)	38	0.77 (0.32-2.31)	54	0.79 (0.33-5.51)	46	0.66 (0.21-1.18)
Chloride mmol/l	37	117.86 (110.00-130.00)	38	115.87 (110.00-126.00)	54	116.13 (104.00-143.00)	46	116.52 (104.00-137.00)
Amylase U/l	37	**690.73ab (387.00-1147.00)**	38	650.61 (358.00-967.00)	54	**583.07a (314.00-1024.00)**	46	**575.57b (322.00-856.00)**
Calcium mmol/l	37	2.42 (1.87-2.87)	38	2.54 (2.06-3.23)	54	2.47 (1.74-2.89)	46	2.53 (1.44-3.63)
Phosphorus mmol/l	37	1.46 (0.48-2.45)	38	1.67 (0.92-3.52)	54	1.46 (0.96-2.64)	46	1.63 (0.73-3.26)
Magnesium mmol/l	37	0.89 (0.69-1.26)	38	0.88 (0.69-1.30)	54	0.84 (0.41-1.15)	46	0.89 (0.71-1.35)
Total protein g/l	37	42.63 (14.80-68.70)	38	46.59 (36.60-65.60)	54	43.76 (27.20-64.20)	46	44.91 (32.80-70.70)
Albumin g/l	37	**16.77a (10.10-23.40)**	38	**19.57a (12.30-29.60)**	54	17.69 (10.70-28.60)	46	18.24 (10.70-31.30)
Glucose mmol/l	37	14.69 (10.27-22.00)	38	13.79 (5.81-19.60)	54	14.65 (9.30-24.90)	46	14.15 (7.06-18.90)
Total bilirubin µmol/l	37	13.85 (8.10-23.80)	38	12.88 (7.30-20.70)	54	12.52 (5.80-23.80)	46	13.11 (5.90-31.50)
Creatinine µmol/l	37	42.89 (35.00-55.00)	38	**44.6a (35.00-52.00)**	54	43.22 (27.00-52.00)	46	**41.91a (31.00-51.00)**
Uric acid µmol/l	37	454.35 (71.00-1208.00)	38	487.26 (136.00-1655.00)	54	439.83 (134.00-712.00)	46	444.28 (134.00-1236.00)

Table 2. Mean, minimum and maximum values of White Storks' blood parameters between different age groups. The same lowercase letters in each blood parameter indicate significant differences revealed by the Tukey's multi comparison test, whereas the different letters - non-significant.

Age	Juv-0		Juvenile		Adult	
Parameter	**n**	**X̄ (min-max)**	**n**	**X̄ (min-max)**	**n**	**X̄ (min-max)**
AST U/I	109	382.65 (100.00-800.00)	34	328.32 (52.00-539.00)	32	388.28 (198.00-1000.00)
ALT U/I	109	100.81 (20.00-536.00)	34	96.35 (39.00-212.00)	32	92.97 (27.00-220.00)
ALP U/I	109	927.18 (85.00-2879.00)	34	1131.32 (70.00-2387.00)	32	908.84 (99.00-3258.00)
LDH U/I	109	1163.52 (210.00-3028.00)	34	1317.59 (667.00-2709.00)	32	1247.50 (667.00-2963.00)
CK U/I	109	1140.23 (236.00-3064.00)	34	1260.29 (741.00-2369.00)	32	1097.84 (245.00-2258.00)
Cholesterol mmol/l	109	**6.56a (1.79-13.30)**	34	**7.52ab (5.03-11.23)**	32	**6.06b (2.31-7.89)**
TG mmol/l	109	0.79 (0.21-5.51)	34	0.72 (0.25-1.02)	32	0.79 (0.33-5.51)
Chloride mmol/l	109	116.34 (104.00-128.00)	34	117.47 (112.00-125.00)	32	116.34 (104.00-143.00)
Amylase U/I	109	618.57 (314.00-1024.00)	34	599.18 (322.00-946.00)	32	628.13 (358.00-1147.00)
Calcium mmol/l	109	2.51 (1.44-3.63)	34	2.5 (1.87-3.23)	32	2.41 (1.78-2.88)
Phosphorus mmol/l	109	**1.58b (0.73-3.52)**	34	**1.67a (0.87-2.45)**	32	**1.32ab (0.48-2.36)**
Magnesium mmol/l	109	**0.86a (0.41-1.35)**	34	**0.94ab (0.69-1.26)**	32	**0.85b (0.66-1.19)**
Total protein g/l	109	**43.89a (24.40-69.30)**	34	**47.38ab (35.20-70.70)**	32	**42.58b (14.80-52.60)**
Albumin g/l	109	17.94 (10.70-28.60)	34	**19.21a (10.70-31.30)**	32	**16.81a (10.10-25.70)**
Glucose mmol/l	109	14.40 (7.06-24.50)	34	13.66 (5.81-19.60)	32	15.05 (10.90-24.90)
Total bilirubin µmol/l	109	13.14 (5.80-31.50)	34	13.56 (8.20-23.80)	32	12.30 (5.90-19.50)
Creatinine µmol/l	109	42.90 (27.00-52.00)	34	43.15 (31.00-55.00)	32	44.00 (35.00-51.00)
Uric acid µmol/l	109	462.35 (134.00-1655.00)	34	410.56 (71.00-639.00)	32	482.41 (175.00-712.00)

Discussion

This is the first biochemistry study of White Storks in Bulgaria. Previously there have been similar evaluations of a number of biochemistry parameters of free-living White Stork chicks (Puerta et al., 1989), free-living White Stork adult birds (Alonso et al., 1991), both wild and captive-bred young White Storks in Spain (Montesinos et al., 1997), 342 chicks from nests in Poland (Jerzak et al., 2010), 80 captive adults from Hungarian zoos (Szabó et al., 2010). It has been previously reported however that biochemistry reference values of captive individuals should not be used for their wild counterparts - many differences could stem from food type and intake, physical activity or the lack of such and so on (Lumeij, 2008). In this case, comparable studies were those describing free-living chicks (Puerta et al., 1989; Montesinos et al., 1997; Jerzak et al., 2010) and free-living adults (Alonso et al., 1991). However, they reported different combinations of parameters and ultimately had different findings.

Location - a parameter which we suspected would make a difference in our values, was reported to significantly affect the albumin values between northwestern and northeastern stork populations, and the creatinine values of northeastern and southeastern populations in Bulgaria. This difference regarding location could be explained by availability of different food items in different regions, however, overall we did not find location to be a factor affecting many of the plasma biochemistry parameters tested of White Storks in Bulgaria. Regarding age, we also did not report many affected parameters - we found a significant difference only in the value of albumin between juveniles and adults, however it was decreasing with age in our case, contrary to Montesinos et al.'s (1997) findings.

Our research may also have some alternative applications. For example, some biochemical parameters can be used in indicating the level of stress of the birds during the time of their handling by humans - it is widely known that glucose plasma concentrations rise during stressful situations. Osadcha et al. (2023) have noted significant AST and ALT rises during stress in chicken. The same study notes increase in creatinine and total bilirubin and decrease in ALT levels during lightning stress (the birds were left without light for 24 hours). Nwaigwe et al. (2020) described rise in albumin levels in broilers during stress periods and changes in uric acid/albumin ratio. This proves that there may be changes in the biochemical profile of birds during stress, which can lead to misunderstanding of their condition during a potential veterinary check-up.

In some cases, biochemical analyses can serve as proof of some sort of pollution. This type of research is done mainly with plants (Shaheen, 2015; Pandit & Sharma, 2020). Biochemical parameters in fish can be used for detecting water pollution (Prabakaran et al., 2010; Mohamed, 2020). While birds' blood parameters cannot serve as a main indicator of environmental pollution, they can give us information of the impact it has on their health and population.

Despite the number of already studied White Storks, we report different findings regarding their biochemistry to the previous studies. Therefore, our study could be used as a base for evaluating the individuals needing veterinary treatment in Bulgaria, as it presents recent data from a relatively big sample size - 175 free-living White Storks.

Conclusions

Establishing biochemistry values is important for health management in wildlife rehabilitation centers. These values vary significantly between species, but also they can be specific to age, location, sex, diet, amongst other factors. There were a number of studies on different groups of White Storks' biochemistry in other countries in Europe, and this is the first one presenting the values of 18 blood biochemistry parameters for White Storks in Bulgaria - some of which were shown to be affected by their region of origin, some by age. It should be noted that sex was not assigned to the 175 individuals, so the results are combined for both. Even so, this is not normally tested upon admittance in wildlife rescue centers, so these results could aid the veterinary specialists dealing with White Storks in the country, and serve as a basis for further studies. Our study can contribute to the improvement of veterinary diagnostics of storks and other wildlife birds at all and establishing a correct database of reference values. It can also help us adapt better rehabilitation approaches, which increases the admitted to the wildlife rescue centers birds' chances of survival, full recovery and their return to their natural habitat.

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