



Blood chemistry in white stork *Ciconia ciconia* chicks varies by sex and age

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ABSTRACT

Little is known on how blood biochemistry differs among avian chicks, especially in sexually monomorphic species. In this study we sampled blood chemistry of 342 white stork *Ciconia ciconia* chicks from nests in western Poland during four years (2005–2008). Special attention was paid to the effect of chick age and sex on blood biochemistry. Since white stork is a monomorphic species, the sex of chicks was established by a molecular technique. Nine blood biochemical parameters were studied: total protein concentration, urea, uric acid, triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). There were sexual differences in total protein, uric acid, cholesterol, HDL and AST. However, total protein and uric acid only differed significantly between sexes if an age effect was included as a covariate in the analysis. Triglycerides decreased significantly, and AST, increased significantly with chick age.

We confirm that blood biochemistry varies with chick age, but we also found significant differences between the sexes. Therefore, to understand changes in blood parameters, and to establish reference ranges useful in captive rearing of this endangered species, establishing gender may be important, even in very young individuals.

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1. Introduction

Blood chemistry parameters have been examined in many bird species and it has been shown that these biochemical variables were related to the physical condition and nutritional status of the individuals, circadian rhythms and growth. They are therefore often used as a description of the health of an organism (Maxwell, 1993; Dawson and Bortolotti, 1997). However, biochemical values of blood are also modified by the age and sex of a bird (Kasprzak et al., 2006, but see: Dawson and Bortolotti, 1997), therefore this additional information can be crucial for a proper interpretation of the obtained results (Gayathri and Hegde 1994; Dawson and Bortolotti, 1997).

Moreover, blood chemistry is strictly connected with haematological parameters and respiratory function in birds. The capacity of a unit volume of blood to carry oxygen should meet different metabolic requirements of birds and thus should result both from bodyweight and from their total energy requirements (Kendeigh et al., 1977; Kostelecka-Myrcha, 1985; Harper and Turner, 2000).

Different parameters are commonly analyzed during studies of avian blood chemistry, because these levels reveal different information on

health status and/or condition of birds. Plasma metabolites related to fat (triglycerides and cholesterol) and protein (total proteins, uric acid and urea) have been previously related to the physiological state of birds, e.g. indicating metabolic functions (Jenni-Eiermann and Jenni, 1998; Ots et al., 1998; Hollmén et al., 2001; Alonso-Alvarez and Ferrer, 2001; Alonso-Alvarez et al., 2002), and plasma enzymes (aspartate aminotransferase AST, and alanine aminotransferase ALT) are commonly used as diagnostic tools in avian medicine. Triglycerides come directly, or via synthesis in the liver, from the diet and their presence in plasma indicates lipid transport to the peripheral tissues. Hence, high plasma levels of this metabolite are indicative of a state of resorption and high body-fat content, while in fasting birds, their levels are expected to be low (Jenni-Eiermann and Jenni, 1998). Cholesterol is involved in the formation of cell membranes, steroids and biliary acids (Griminger, 1986). A decrease in this compound might be explained by a decline in anabolic processes, and has been found to be a good indicator of body-mass loss below the individual's optimum (Alonso-Alvarez et al., 2002). Circulating levels of proteins in the blood are thought to be an index of total protein reserves (Allison, 1955). Increase in plasma levels of uric acid and urea may reflect mobilization of protein reserves during long term food-shortage periods in birds (Alonso-Alvarez and Ferrer, 2001). In addition, high levels of uric acid may indicate a rich protein diet or dehydration (Hochleithner, 1994), and low levels can indicate short term food stress (Jenni-Eiermann and Jenni, 1994). Clinical enzymology

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provides valuable information on pathological tissue damage, since abnormal leakage of intracellular enzymes from tissues results in increased circulating levels of plasma enzymes (Hernández et al., 1990).

Although there are increasing numbers of papers focussing on clinical avian blood biochemistry, information on the range of variation, especially for different sexes, are scarce for wild populations. Obviously, there exist a number of papers that have analyzed sexual differences in blood biochemistry in adults, sometimes also among chicks, but for those species with strong sexual dimorphism, even where sometimes reversed as in raptors (Bowerman et al., 2000; Müller et al., 2001; Casado et al., 2002; Blas et al., 2006). However, only a minority of bird species show sexual dimorphism during the nestling stage. A good example is the white stork *Ciconia ciconia*, a monomorphic large species, a migratory soaring bird in eastern and central Europe, and an icon of nature conservation in many countries. Information on the condition and health status of individuals was suggested as an important tool for conservation of this particular species, and used both in rehabilitation centres, and for reintroduction projects (Schaub et al., 2004; Olsson, 2006). To date, blood chemistry has been analyzed in the white stork (Montesinos et al., 1997) and even an age effect (but only a comparison of chicks versus adults) on blood parameters was discussed (Blas et al., 2006). However, the novelty of our paper is incorporating the effect of the sex of the chick into blood chemistry analysis.

Similar to other species (Bowerman et al., 2000; Shmueli et al., 2000; Gayathri et al., 2004; Villegas et al., 2004), we predict that variation in blood parameters between males and females will increase with age, mainly due to an increase in activity of sex hormones. Therefore, in the present work we present values of nine plasma metabolites and enzyme activities (total protein concentrations, uric acid, urea, total cholesterol and two different fractions high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL), triglycerides (TG), alanine aminotransferase (ALT), and aspartate aminotransferase (AST)) of nestling white storks in the wild, examining the influence of age and sex on these variables.

2. Material and methods

Studies were performed in 2005–2008 in Western Poland in an area between 51°40′–54°38′ N and 14°42′–17°33′ E, in habitats with a relatively high density of the white stork (for more details see: Tryjanowski et al., 2005; Kulczykowska et al., 2007). All white storks bred in open nests on roofs, at the top of trees, or on electricity posts. Chicks hatch after approximately one month of incubation, but rely on both parents for food and protection during the next 70–80 days. Our studies were carried out during their nesting season, between 23rd June and 4th July in each year.

Blood samples (5 mL each) were collected from 342 nestlings via a veni-puncture of the brachial vein. Each chick was checked to establish physical condition by general behaviour, color, weight and fat on the breast muscle, and if satisfactory was removed from the nest

and placed in individual ventilated cotton sacks. Age was estimated by measurement of the bill length according to Kania (1988). The mean (\pm SE) age of chicks during sampling was 35.3 ± 1.43 days, when white stork nestlings display very little response to capture and blood sampling (Blas et al. 2006).

Nine variables, including enzymatic activities, were determined for each sample using a multiparametric autoanalyser (Cobas Mira, Roche, www.roche-applied-science.com) with the reagents recommended by Cormay (www.pzcormay.pl). These were: total protein concentrations, uric acid, urea, cholesterol – total level, and two different fractions HDL and LDL, triglycerides, alanine aminotransferase (EC: 2.6.1.2), and aspartate aminotransferase (EC 2.6.1.1). All determinations were performed at 37 °C. In some cases, the amount of plasma obtained was insufficient to assay all the compounds, therefore the sample sizes are not uniform.

As white storks do not exhibit strong sexual dimorphism we resorted to molecular sexing of the birds by using the cellular fraction of the blood as a source of DNA (Fridolfsson and Ellegren, 1999).

Data are analyzed and presented in two ways. This was thought necessary because of the dominance of male chicks in terms of both number (56% overall) and age (mean 38.8 (range 18–62) days versus 35.0 (range 15–51) days for females). First, study parameters are presented as unadjusted means \pm SE and sample size, and means of female and male chicks are compared with a two sample *t*-test. Secondly, a more robust analysis using a nested ANOVA with covariates was used to compare for sex differences. The model included year as a factor, brood nested within year, and age as a covariate, thus focussing on within brood differences adjusted by age. Statistical analyses were conducted using the Minitab v.15 package (www.minitab.com). The range of blood parameters is shown for each sex as the 2.5th and 97.5th percentile of each blood parameter.

3. Results

A total of 342 nestling white storks (192 males and 150 females) were studied. All birds appeared to be in good condition, and no abnormalities were noted during physical examination or in blood chemistry analyses. Significant sex-dependent differences were observed in total protein level, uric acid, cholesterol, HDL and AST; the first two only if an age effect was controlled in the analysis (Tables 1 and 2, Figs. 1 and 2). Moreover, TG increased significantly, and AST decreased significantly with age of chicks within a nest (Table 2, Fig. 2). Nested ANOVA models eliminating year-to-year effects, differences between nests, and age and sex effects explained from 65.1 to 90.4% of the total variation in the blood parameters (Table 2).

4. Discussion

Birds have special adaptations for preventing age-related tissue damage caused by haematological and biochemical changes in their

Table 1

2.5th and 97.5th percentiles and unadjusted mean blood chemistry values of female and male white stork chicks, the latter compared using a 2 sample *t*-test. Rows in bold indicate statistical significance in means between sexes.

	Female chicks					Male chicks					2 sample <i>t</i> -test	
	<i>n</i>	2.5	97.5	Mean	SE	<i>n</i>	2.5	97.5	Mean	SE	<i>t</i>	<i>P</i>
Protein (g/L)	144	28.5	46.4	37.4	0.4	189	27.7	45.2	36.8	0.3	1.21	0.226
Urea (mmol/L)	143	1.12	4.44	2.40	0.07	188	1.11	4.35	2.27	0.06	1.41	0.160
Uric acid (μ mol/L)	144	270	1288	721	26	189	251	1245	679	22	1.22	0.222
Triglycerides (mmol/L)	144	0.77	5.03	1.78	0.10	188	0.73	4.40	1.72	0.07	0.55	0.583
Cholesterol (mmol/L)	144	3.55	7.06	5.09	0.08	189	3.39	6.72	4.84	0.06	2.72	0.007
HDL (mmol/L)	142	1.50	3.14	2.29	0.03	188	1.52	2.99	2.14	0.03	3.47	0.001
LDL (mmol/L)	142	0.93	3.54	1.99	0.05	183	0.77	3.88	1.93	0.05	0.86	0.389
AST (U/L)	144	155	284	221	3	189	136	258	198	2	6.35	<0.001
ALT (U/L)	144	17.6	57.0	38.0	0.8	189	16.8	59.0	37.4	0.7	0.57	0.569

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