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ORIGINAL ARTICLE

Haematocrit and blood biochemical parameters in free-living Eurasian eagle owls (*Bubo bubo*) from Southeastern Spain: study of age and sex differences

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Abstract The study of haematocrit and biochemical parameters is considered a useful tool for diagnosis of health status in birds. However, an adequate interpretation requires reference values for each species. Moreover, factors such as age and sex should also be taken into account, as it is well known that they can also influence several parameters. For these reasons, serum biochemical parameters (albumin, calcium, phosphorus, aspartate aminotransferase, alkaline phosphatase, cholesterol, triglycerides, creatine kinase, γ-glutamyltransferase, glucose, lactate dehydrogenase, total proteins and uric acid) were studied in 15 adults (6 males and 9 females) and 258 chicks of free-living Eurasian eagle owl (*Bubo bubo*), one of the owls with the largest distribution range worldwide. Most blood clinical parameters were higher in nestlings than in adults, but only significantly for alkaline phosphatase, phosphorus and uric acid. On the contrary, γ-glutamyltransferase was significantly lower in nestlings. In adults, sex differences were not significant. Haematocrit was studied in 13 adults (2 males and 11 females) and 162 nestlings, and levels were significantly higher in adults than in nestlings. Because samples were obtained from healthy free-living individuals and the values fall within the range of levels considered physiological for similar species, we suggest that can be used as reference values for Eurasian eagle owls.

Keywords Serum · Biochemistry · Owls · Free-range · Haematocrit · Reference values

Introduction

The study of biochemical parameters is considered a useful tool to evaluate the general health and the effects of a specific therapy. For example, increased activities of aspartate aminotransferase and γ-glutamyltransferase are, in general, indicative of liver damage, while uric acid is measured to monitor renal disease. In the case of birds, where clinical signs of illness are frequently subtle, the analysis of these parameters can be more necessary and especially important to evaluate changes at cellular level (Hochleithner 1994). An adequate interpretation of results demands for the availability of reference values for each species and, ideally, each physiological status, since these factors, as well as age, sex, circadian rhythms or diet, may influence several of these parameters (Joseph 1999; Villegas et al. 2002; van Wyk et al. 1998). For these reasons, interpretation of blood biochemistry requires a large

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database for comparison (Wolf et al. 1985). Nevertheless, the majority of parameters should fall within certain limits which have been considered characteristic for each species (de le Court et al. 1995) or at least, for a group of related species (Bairlein 1996; Ferrer et al. 1987). In spite of the growing interest in knowing the normal values and range of variation of blood biochemical parameters in wild birds (Villegas *et* al. 2002), this type of information is still scant, and results are usually obtained by a diversity of techniques, which makes the comparison among studies even more difficult (Ammersbach et al. 2015; Gómez-Ramírez et al. 2011; Harr 2006; Hochleithner 1994; Martínez-López et al. 2004). In addition, samples are usually taken from animals held in captivity, and the studies concerning wild animals, especially raptors, are still anecdotic (Casado et al. 2002; Johnson et al. 2009; Sonne et al. 2012; van Wyk et al. 1998; Villegas et al. 2002). The information regarding the condition and health of animals as individuals can be greatly useful both in rehabilitation centres and for reintroduction projects and is considered an important tool for the conservation of species (Olsson 2006; Schaub et al. 2004). For these reasons, the study of blood parameters using samples from free-range animals of different species is of interest.

The Eurasian eagle owl (*Bubo bubo*) is the biggest nocturnal raptor in Spain, where it is sedentary and highly territorial during the whole year, occupying territories whose size and foraging area depend on the prey availability. Around the world, this species has one of the largest ranges of any eagle owl (Warhol, 2007), being found across much of Europe, through the Middle East, Russia and Asia, and as far as East China, Korea and Japan (del Hoyo et al. 1999; Weick 2006). Although generally absent in Britain and Ireland (Weick 2006), small numbers are now beginning to breed in Britain (Melling et al. 2008). On the contrary, the Eurasian eagle owl population in the region of Murcia and Alicante is abundant and estimated to be approximately 470–530 pairs (Martínez and Calvo 2006; Pérez-García and Sánchez-Zapata 2015) and shows the highest breeding density described within all its distribution range (Pérez-García et al. 2011). Besides, productivity of this population is much higher than in other populations studied in the whole Palearctic (Marchesi et al. 2002).

Due to the relative abundance of this species worldwide, the availability of a database on serum metabolites, enzyme activities and inorganic ions of free-living nestlings and adults Eurasian eagle owls can be of interest for the conservation of this species, being useful in rehabilitation centres and reintroduction programs. Hence, the main aim of our study was to increase this database, using the greatest number of samples collected from wild nestlings and adults of both sexes. The influence of factors such as age and sex on these parameters was also evaluated.

Material and methods

Blood sampling

Blood samples were obtained between 2003 and 2012 from nestlings and adults Eurasian eagle owls from the mountains Escalona, Altaona, Monte el Valle, Columbares, La Muela-Cabo Tiñoso and Almenara (37°45′ N, 0°57′ W), in the south of the province of Alicante and the east of Murcia Region, in South-eastern Spain (Fig. 1).

Reproductive activity was rigorously monitored from December until fledging, to determine the precise age of the nestlings and get blood samples when chicks were 28–30 days old. Adults were trapped in their territories using two methods described by Penteriani et al (2010): (a) simulating a territorial intrusion with a combination of a stuffed mount of a Eurasian eagle owl and a net for males and (b) using a bownet placed in the nest when nestlings were 20–35 days old (i.e. when they were already able to thermoregulate) for females. These adult individuals were aged and sexed according to their moult pattern (Martínez et al. 2002) or the date that were ringed as chicks.

To avoid stress in the animals, careful steps were taken, like covering the head of the individuals during manipulation. Blood samples were obtained by the veterinarian of the Wildlife Rehabilitation Centre 'Santa Faz' (Alicante, Spain), who evaluated clinically the health status of the owls prior to sampling. This clinical exploration includes the evaluation of general body conformation, posture, attitude, stimulus response and character of respiration. Also, it includes exploration of the feathers, skin, beak, eyes, ears, cere, nares, oral cavity, bones, muscles (particularly breast muscle), wings, faeces, abdomen and vent. Between 3 and 5 mL of blood were taken by puncturing the brachial vein with a 23G needle and a syringe. Samples were placed in tubes without anticoagulant and immediately taken to the laboratory under refrigerated conditions where they were centrifuged at 5000 g for 5 min. Serum samples were transferred to Eppendorf® tubes and kept frozen between −40 and −80 °C until analysis.

Albumin, calcium, phosphorus (P), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol, triglycerides (TGL), creatine kinase (CK), γ-glutamyltransferase (γ-GT), glucose, lactate dehydrogenase (LDH), total proteins (TP) and uric acid were analysed in 273 serum samples (15 adults—6 males and 9 females—and 258 chicks, obtained from 2003–2010) using an automated spectrophotometric analyser (A25, Atom®). Techniques used to determine each parameter are shown in supplementary information (SI; Table SI1). Every 40 samples, control samples provided by the kit manufacturer were analysed to check that the results were within the certified range. Haematocrit was determined in 13 adults (2 males and 11 females) and 162 chicks (sampled from 2009–2012) centrifuging microcapillaries at 2200 g for 5 min and using a microhaematocrit reader.

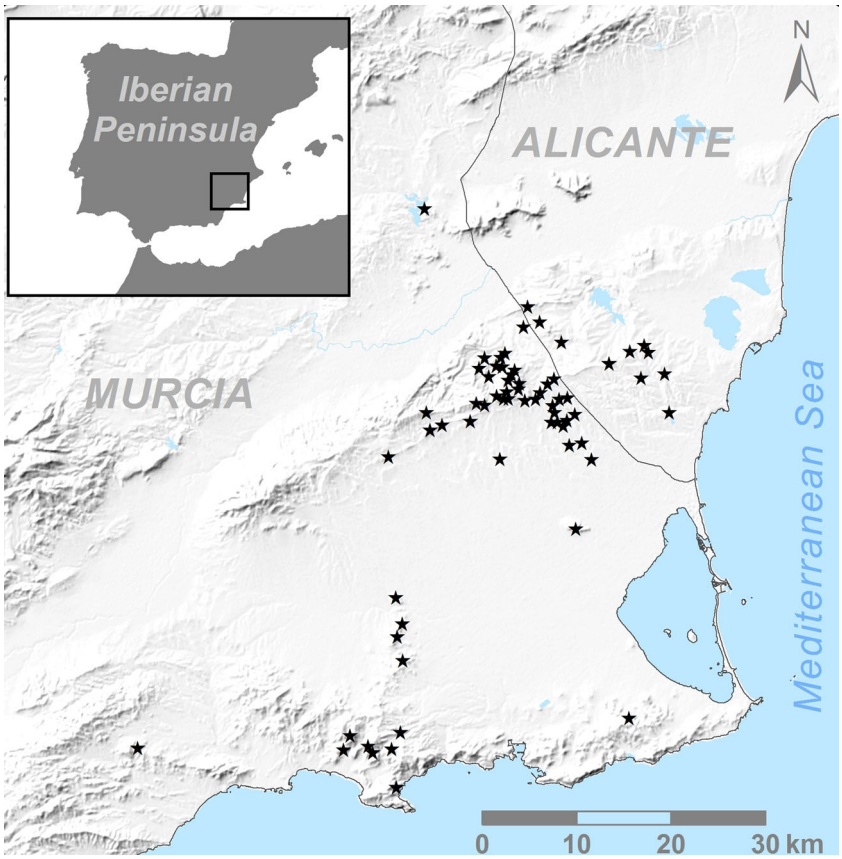

Fig. 1 Location of the study area (province of Alicante and Murcia Region) in Southeastern Spain. *Black stars* indicate sampled nests

A greater number of samples were obtained. However, haemolytic samples were ruled out in order to avoid interferences in certain parameters such as ALP, LDH, AST, calcium, albumin or CK. Similarly, because some contaminants such as metals and organochlorine insecticides can alter biochemical parameters (Dieter and Wiemeyer 1978; Mateo et al. 2003), samples from animals suspicious to be exposed to pollutants were not included. The selection was based on the fact that most samples had been analysed for organochlorine pesticides (α-HCH, β-HCH, δ-HCH, γ-HCH, aldrin, dieldrin, endrin, endosulfan I, endosulfan II, p,p'-DDT, p,p'-DDD, p,p'-DDE, heptachlor and heptachlor epoxide; Gómez-Ramírez 2011) and metals (lead, cadmium, zinc, copper and mercury; Espín et al. 2014a, b; Gómez-Ramírez et al. 2011). Although the levels of these contaminants were considered too low to cause acute health effects, samples with total organochlorine pesticides levels >5 μg/mL and/or with lead levels >5 μg/dL were ruled out. This limit was chosen because no significant correlation between organochlorine pesticides and/or metals and the blood parameters studied was found under these levels (Gómez-Ramírez et al. in prep). Similarly, the limit of 3 μg/dL of mercury was chosen according to the effects on oxidative stress found by Espín et al. (2014a) in this population.

Statistical analyses

Statistical analyses were performed using SPSS v15.0 statistical software (SPSS Inc., 1989–1999). Reported biochemical parameters represent the median, mean ± standard deviation and range. Repeated measures ANOVA and paired *t* test were used in order to test differences between siblings of the same nest. Since no significant differences were found, the average value of each nest was used as unit to compare the parameters between adults and nestlings. Normality was checked using Shapiro-Wilk test. Sex and age-related differences in biochemical parameters and age-related differences in haematocrit were checked using Mann-Whitney test as biochemical parameters were not normally distributed. The level of significance was set at $\alpha = 0.01$. To estimate the magnitude of biologically relevant differences independently of sample size (Nakagawa and Cuthill 2007), effect size was calculated in those variables that were significantly different between nestlings and adults, according to Rosenthal (1991).

Results

To our knowledge, this is the first report of serum biochemical parameters and haematocrit for free-living Eurasian eagle

owls, both nestlings and adults. Results are shown in Tables 1 and 2. Values found in other birds of prey to be compared with our samples are shown in SI (Tables SI2 and SI3). Sex-related differences in biochemical parameters were studied among adults (Table 1), but no significant differences were found. Haematocrit was not included in this analysis because it could only be determined in two males.

Most biochemical parameters were higher in nestlings than in adults (Table 2), although, according to Mann-Witney test, the difference was only significant for ALP (median 550.60 vs 19.62 U/L; $p < 0.01$, large size effect: $r = -0.62$), P (median 7.24 vs 2.01 mg/dL; $p < 0.01$, large size effect: $r = -0.58$) and uric acid (median 12.39 vs 7.22 mg/dL; $p < 0.01$, medium size effect: $r = -0.37$). On the contrary, haematocrit and γ-GT values were significantly lower ($p < 0.01$) in nestlings than in adults (median haematocrit 28.7 vs 43.67 %, large size effect: $r = -0.56$; median γ-GT 0.44 vs 3.51 U/L, large size effect: $r = -0.51$).

Discussion

The correct interpretation of haematological values requires the availability of well-established normal reference values. Unfortunately, many species have poorly defined 'normal' ranges, and information regarding physiological or clinical status is lacking for many studies in birds of prey. In addition, published reference ranges may have been obtained by different methods, and therefore, may not be directly comparable (Monks and Forbes 2007).

As shown in Table SI2, only four studies concerning biochemical parameters in captive adult Eurasian eagle owls were found (Ammersbach et al. 2015; Ferrer et al. 1987; García-Rodríguez et al. 1987; Jennings 1996). For this reason, the values from our adults were also compared with data from other captive owl species like Great horned owl (*Bubo virginianus*), Tawny owl (*Strix aluco*), Eastern screech owl (*Megascops asio*) or Barn owl (*Tyto alba*) (Altman et al. 1997; Ferrer et al. 1987; Franson et al. 1985; Spagnolo et al.

Table 1 Median, mean ± SD (range) of biochemical parameters in males and females of adult Eurasian eagle owls in this study

	Males	Females
Haematocrit (%)	46.50; 50 $n = 2$	42.00; 42.67 ± 4.02 (34.00–50.00) $n = 11$
Albumin (g/L)	16.68; 16.78 ± 2.29 (13.82–20.44) $n = 6$	17.43; 16.08 ± 4.42 (6.31–20.56) $n = 9$
Alkaline Phosphatase (U/L)	19.62; 20.60 ± 6.39 (14.72–29.43) $n = 5$	19.62; 20.70 ± 5.35 (11.07–29.68) $n = 9$
Aspartate aminotransferase (U/L)	217.41; 192.27 ± 98.88 (28.28–285.16) $n = 6$	169.68; 199.79 ± 101.42 (50.74–362.94) $n = 9$
Calcium (mg/dL)	10.91; 10.99 ± 1.91 (8.83–13.70) $n = 6$	8.92; 8.65 ± 0.93 (7.11–10.25) $n = 9$
Cholesterol (mg/dL)	193.73; 191.27 ± 36.43 (148.83–233.70) $n = 6$	185.73; 189.57 ± 26.53 (136.53–223.00) $n = 9$
Creatine kinase (U/L)	1440.12; 1376.11 ± 823.55 (322.03–2302.19) $n = 4$	1052.08; 1028.45 ± 551.01 (466.00–1567.26) $n = 3$
γ-glutamyltransferase (U/L)	2.23; 2.38 ± 1.37 (0.79–4.02) $n = 5$	0.98; 1.02 ± 0.6 (0.44–1.64) $n = 3$
Glucose (mg/dL)	403.54; 406.74 ± 88.43 (252.37–497.05) $n = 6$	415.01; 395.81 ± 67.69 (265.18–466.31) $n = 9$
Lactate dehydrogenase (U/L)	836.62; 1111.94 ± 426.00 (792.38–1726.53) $n = 5$	790.19; 753.68 ± 297.25 (410–1201.68) $n = 5$
Phosphorus (mg/dL)	2.86; 3.46 ± 2.64 (1.31–8.61) $n = 6$	1.81; 2.09 ± 0.99 (0.73–3.91) $n = 9$
Total proteins (g/L)	35.28; 34.44 ± 5.43 (24.19–39.31) $n = 6$	32.9; 31.13 ± 11.59 (4.03–43.35) $n = 9$
Triglycerides (mg/dL)	90.37; 85.69 ± 21.52 (44.55–105.64) $n = 6$	108.18; 115.76 ± 40.65 (42.00–170.02) $n = 9$
Uric acid (mg/dL)	7.12; 8.15 ± 3.54 (3.59–12.68) $n = 6$	7.64; 9.02 ± 4.15 (4.00–16.86) $n = 9$

Table 2 Median, mean ± SD (range) of biochemical parameters and haematocrit in plasma of adults and nestlings of Eurasian eagle owl from Southeastern Spain. Asterisks indicate significant differences ($p < 0.01$) between adults and nestlings, which were calculated using the average of each nest

	Adults	Nestlings
Haematocrit (%)	44.41; 43.53 ± 4.29*	29.00; 29.71 ± 5.84
	(34–50) $n = 13$	(14–49) $n = 162$
Albumin (g/L)	17.43; 16.36 ± 3.63	16.53; 16.35 ± 2.33
	(6.31–20.56) $n = 15$	(3.61–21.94) $n = 254$
Alkaline Phosphatase (U/L)	19.62; 20.66 ± 5.5*	527.30; 549.95 ± 165.32
	(11.07–29.68) $n = 14$	(18–1162.51) $n = 242$
Aspartate aminotransferase (U/L)	201.50; 196.78 ± 96.87	179.11; 195.65 ± 76.88
	(28.28–362.94) $n = 15$	(15.32–471.35) $n = 257$
Calcium (mg/dL)	9.03; 9.59 ± 1.79	10.34; 10.28 ± 1.95
	(7.11–13.70) $n = 15$	(4.76–20.89) $n = 256$
Cholesterol (mg/dL)	185.73; 190.25 ± 29.61	205.41; 208.13 ± 35.50
	(136.53–233.70) $n = 15$	(114.39–313.65) $n = 256$
Creatine kinase (U/L)	1270.10; 1227.11 ± 689.10	460.04; 1029.45 ± 1046.23
	(322.03–2302.19) $n = 7$	(10–3438.28) $n = 208$
γ-glutamyltransferase (U/L)	1.50; 1.87 ± 1.29*	3.73; 4.4 ± 5.34
	(0.44–4.02) $n = 8$	(0.11–68) $n = 210$
Glucose (mg/dL)	411.22; 400.18 ± 73.77	438.13; 437.21 ± 47.22
	(252.37–497.05) $n = 15$	(183.19–552.14) $n = 255$
Lactate dehydrogenase (U/L)	801.43; 932.81 ± 394.44	815.5; 845.32 ± 321.34
	(410.00–1726.53) $n = 10$	(141.78–1967.87) $n = 229$
Phosphorus (mg/dL)	2.01; 2.63 ± 1.88*	7.13; 7.29 ± 1.37
	(0.73–8.61) $n = 15$	(5.07–14.58) $n = 255$
Total proteins (g/L)	34.27; 32.46 ± 9.49	32.26; 31.62 ± 4.60
	(4.03–43.35) $n = 15$	(6.14–41.33) $n = 258$
Triglycerides (mg/dL)	98.00; 103.74 ± 36.63	124.09; 127.19 ± 44. 09
	(42.00–170.02) $n = 15$	(25.45–285.09) $n = 256$
Uric acid (mg/dL)	7.22; 8.67 ± 3.81*	12.29; 12.84 ± 4.73
	(3.59–16.86) $n = 15$	(1.05–30.31) $n = 256$

2008; Table SI2). According to this, levels of biochemical parameters in our individuals were in the same range as in the mentioned species, except for P, TGL and LDH. P blood levels can be influenced by diet (Harr 2006). This could explain the fact that P levels in our adults are lower than in the individuals from the compared studies (García-Rodríguez et al. 1987; Altman et al. 1997; Spagnolo et al. 2008), where animals were held in captivity. Similarly, TGL levels were higher in the Eurasian eagle owls studied by García-Rodríguez et al. (1987). Increased TGL have been related to stressful conditions, such as handling (Thaxton and Puvadolpirod 2000), although more information about this parameter in owls would be needed. Similarly to TGL, to our knowledge, LDH in adult Screech and Barn owls was only analysed by Franson et al. (1985). LDH levels were lower, even half than in adults of our study. Although serum LDH can be a biomarker for liver disease in birds, the wide reference interval of this enzyme in healthy birds makes it less sensitive than other enzymes (i.e. ALT and AST) for this purpose (Campbell 1987).

Some studies have found differences between male and female adult birds. For example, pre-egg laying and laying mallards did show significant differences in several serum parameters compared to males (Fairbrother et al., 1990). In fact, within each sex, values from all the parameters differed among reproductive conditions, although the pattern of change was not the same for both sexes. Although not statistically significantly, calcium levels in males were higher than in females in our study. Our samples were taken during breeding season, after hatching of nestlings. Oestrogen induces dramatic increases in blood total calcium concentration in reproductive, oviparous females (Simkiss, 1967). These higher levels of calcium may come from the medullary bone, which means a freely available storage depot of calcium in female birds (Johnston and Ivey, 2002). After egg laying, calcium from medullary bone should be replaced. This could be the reason for a decrease in serum calcium levels, and thus, for the difference between genders found in our study. Further studies with greater number of samples may be able to find significant differences in biochemical parameters between sexes.

Unfortunately, biochemical parameters have not been previously studied in nestling owls. Hence, our data were compared with nestling Northern goshawks (*Accipiter gentilis*), Golden eagles (*Aquila chrysaetos*) and White-tailed eagles (*Haliaeetus albicilla*) (Sonne et al. 2012), Ospreys (*Pandion haliaetus*) (Muriel et al. 2013), Booted eagles (Casado et al., 2002), Bald eagle (*Haliaetus leucocephalus*) (Mealey et al. 2004) and Spanish imperial eagles (*Aquila adalberti*) (Ferrer and Dobado-Berrios 1998; Hoefle et al. 2000). Most biochemical parameters in our nestlings were in the same range as in the mentioned studies (Table SI3).

As mentioned above, age is an important factor that can influence some biochemical parameters, and several authors have found significantly higher values in young birds than in adults (Fairbrother et al. 1990; Viñuela et al. 1991; Puerta et al. 1992; Polo et al. 1994; de le Court et al. 1995; Stout et al. 2010). For example, activity of ALP (an enzyme induced by increased cellular activity) is higher in growing individuals, since this enzyme is synthesised by osteoblasts (Dobado-Berrios and Ferrrer 1997). In fact, as shown in Table 2, ALP activity can be 10 to 100 times higher in nestlings than in adults. In this sense, when the activity of this enzyme in nestlings of Spanish imperial eagle was compared with the activity in adults, ALP was found to be significantly higher in the nestlings (1295 ± 47 vs 64 ± 28 U/L) (Dobado-Berrios and Ferrrer 1997). Hence, although elevations of ALP have been related to liver disease, enteritis or hyperthyroidism-induced stimulation of osteoplastic activity (Hochleithner 1994), the activity in our nestlings seems to be physiological as it is also similar, or even lower, to the activity in other nestlings of different species of birds of prey (Sonne et al. 2012). P levels are also usually decreasing with age as it is involved in bone and muscle development (Clubb et al. 1991a, b, c; Wolf et al. 1985) and were also higher in Spanish imperial eagle nestlings than in adults (6.7 ± 0.3 vs 3.2 ± 0.3 mg/dL) studied by Dobado-Berrios and Ferrrer (1997) and in Red kites (*Milvus migrans*) (nestlings 9.41 ± 3.46 mg/dL; adults 1.92 ± 0.77 mg/dL) and Black kites (*Milvus milvus*) (nestlings 8.52 ± 3.78 mg/dL; adults 3.5 ± 2.21 mg/dL) studied by Viñuela et al. (1991). Another consequence of growing status is an increase in protein synthesis, which causes an increase in uric acid (Hochleithner 1994). However, these higher levels in uric acid can also be related to the larger daily protein intake of chicks (Griminger and Scanes 1986), since uric acid is the main nitrogen waste in birds (e.g. Singer 2003). γ-GT is a peptidase enzyme that is considered a biomarker of liver disease in mammals, but its role in birds is still under discussion. Nevertheless, compared to other studies in birds of prey, levels are usually lower in nestlings (see tables SI2 and SI3), which coincides with our results. On the other hand, the small effect size in this parameter ($r = -0.07$) suggests that a greater number of adults would be needed to corroborate this finding.

Haematocrit, or packed cell volume, is a measure of the relative volume of red blood cells compared with total blood volume (Harrison and Harrison 1986). Although this value has frequently been used as an indicator of condition in wild birds, several studies have shown that changes in haematocrit could be caused by a number of different natural factors that include age, sex, geographical elevation, energy expenditure, parasitism, nutrition and genetics (Fair et al. 2007). Haematocrits are usually lower in young individuals than in adults in several animal taxa, including birds of prey (Lanzarot et al. 2001; Provencher Bolliger et al. 2010; Villegas et al. 2002). This could be due to the higher oxygen affinity of foetal haemoglobin, which decreases during development, both in mammals and birds (Bartels et al. 1966). Nevertheless, haematocrit values, both in adults and chicks, were in the same range as in other birds of prey, including owls (Jennings 1996; Ferrer et al. 1987; Fourie and Hattingh 1983; Hawkey and Samour 1988; Spagnolo et al. 2008); thus, we can consider our values as reference for the Eurasian eagle owl.

Admission of Eurasian eagle owls in rehabilitation centres can be quite frequent especially in areas where this species is abundant, like in Southeastern Spain (Generalitat Valenciana 2011). Electrocution in power lines followed by human persecution and collisions with car and fences is the main cause for admission in these centres (Martínez et al. 2006; Molina-López et al. 2011). To rehabilitate these owls, the knowledge of normal concentrations of blood constituents is of paramount importance (Polo et al. 1992). Nevertheless, because reference values for clinically normal free-living individuals are scarce, the interpretation of haematological data relies on values from captive specimens (Averbeck 1992). The difficulty in collecting useful data from free-ranging individuals and the fact that wild bird medicine is of little commercial interest leads to a scarcity of reference values in these species. This makes the diagnosis and the performance of routine laboratory tests difficult, especially in wildlife rehabilitation centres (Gelli et al. 2009). For these reasons, the study of biochemical parameters in free-ranging animals, such as the present study, is remarkable.

Conclusions

The results of this study provide new information for haematocrit and biochemical parameters in Eurasian eagle owls, taking into account factors such as age and sex. Because samples were obtained from healthy free-living individuals and the values obtained fall within the range of levels considered physiological for similar species, we can suggest that these can be used as reference values for free-living Eurasian eagle owls admitted in rehabilitation centres.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

References

Altman RB, Clubb SL, Dorrestein GM, Quesenberry K (1997) Appendix I. In: Altman RB, Clubb SL, Dorrestein GM, Quesenberry K (eds) Avian medicine and surgery. WB Saunders, Philadelphia, pp 1005–1008

Ammersbach M, Beaufrère H, Gionet Rollick A, Tully T (2015) Laboratory blood analysis in Strigiformes-Part II: plasma biochemistry reference intervals and agreement between the Abaxis Vetscan V2 and the Roche Cobas c501. Vet Clin Pathol 44:128–140. doi:10.1111/vcp.12230

Averbeck C (1992) Haematology and blood chemistry of healthy and clinically abnormal great black-backed gulls (*Larus marinus*) and herring gulls (*Larus argentatus*). Avian Pathol 21:215–223

Bairlein F (1996) Fruit-eating in birds and its nutritional consequences. Comp Biochem Physiol 113A:215–224

Bartels H, Hiller G, Reinhardt W (1966) Oxygen affinity of chicken blood before and after hatching. Respir Physiol 1(4):345–356

Campbell T (1987) Avian chemistries. AAV Today 1:121–122

Casado E, Balbontin J, Ferrer M (2002) Plasma chemistry in booted eagle (*Hieraaetus pennatus*) during breeding season. Comp Biochem Physiol 131A:233–241

Clubb S, Schubot R, Joyner K (1991a) Hematological and serum biochemistry reference intervals in juvenile cockatoos. J Avian Med Surg 5:5–16

Clubb S, Schubot R, Joyner K (1991b) Hematological and serum biochemistry reference intervals in juvenile Eclectus parrots (*Eclectus roratus*). J Avian Med Surg 4:218–225

Clubb S, Schubot R, Joyner K (1991c) Hematological and serum biochemistry reference intervals in juvenile macaws. J Avian Med Surg 5:154–162

de le Court C, Aguilera E, Recio F (1995) Plasma chemistry values of free-living white spoonbills (*Platalea leucorodia*). Comp Biochem Physiol 112A:137–141

del Hoyo J, Elliott A, Sargatal J (1999) Barn-owls to Hummingbirds, vol 5. Lynx Edicions, Barcelona

Dieter MP, Wiemeyer SN (1978) Six different plasma enzymes in bald eagles (*Haliaeetus leucocephalus*) and their usefulness in pathological diagnosis. Comp Biochem Physiol 61(C):153–155

Dobado-Berrios P, Ferrrer M (1997) Age-related changes of plasma alkaline phosphatase and inorganic phosphorus, and late ossification of the cranial roof in the Spanish imperial eagle (*Aquila adalberti* C. L. Brehm, 1861). Physiol Zool 70(4):421–427

Espín S, Martínez-López E, León-Ortega M, Martínez JE, García-Fernández AJ (2014a) Oxidative stress biomarkers in Eurasian eagle owls (*Bubo bubo*) in three different scenarios of heavy metal exposure. Environ Res 131:134–144

Espín S, Martínez-López E, León-Ortega M, Calvo JF, García-Fernández AJ (2014b) Factors that influence mercury concentrations in nestling Eagle owls (*Bubo bubo*). Sci Total Environ 470–471:1132–1139

Fair J, Whitaker S, Pearson B (2007) Sources of variation in haematocrit in birds. Ibis 149:535–552

Fairbrother A, Craig MA, Walker K, O'Loughlin D (1990) Changes in mallard (*Anas platyrhynchos*) serum chemistry due to age, sex, and reproductive condition. J Wildl Dis 26(1):67–77

Ferrer M, Dobado-Berrios P (1998) Factors affecting plasma chemistry values of the Spanish Imperial Eagle, *Aquila adalberti*. Comp Biochem Physiol 120A:209–217

Ferrer M, García-Rodríguez T, Carrillo JC, Castroviejo J (1987) Hematocrit and blood chemistry values in captive raptors (*Gyps fulvus, Buteo buteo, Milvus migrans, Aquila heliaca*). Comp Biochem Physiol 87A(4):1123–1127

Fourie LR, Hattingh J (1983) Comparative haematology of some South African birds. Comp Biochem Physiol 74A:443–448

Franson JC, Murray HC, Bunck C (1985) Enzyme activities in plasma, kidney, liver, and muscle of five avian species. J Wildl Dis 21:33–39

García-Rodríguez T, Ferrer M, Recio F, Castroviejo J (1987) Circadian rhythms of determined blood chemistry values in buzzards and eagle owls. Comp Biochem Physiol 88A:663–669

Gelli D, Ferrari V, Franceschini F, Lai O, Laricchiuta P, Zanella A, Bernardini D, Romagnoli S (2009) Serum biochemistry and electrophoretic patterns in the Eurasian buzzard (*Buteo buteo*): reference values. J Wildl Dis 45(3):828–833

Generalita Valenciana (2011) Balance de actividades 2011, Centro de Recuperación de Fauna Santa Faz. Conselleria d'Infraestructures, Territori i Medi Ambient

Gómez-Ramírez P (2011) The Eagle Owl (*Bubo bubo*) as biomonitor of persistent environmental pollutants in Southeastern Spain. Dissertation. University of Murcia

Gómez-Ramírez P, Martínez-López E, María-Mojica P, León-Ortega M, García-Fernández AJ (2011) Blood lead levels and δ-ALAD inhibition in nestlings of Eurasian eagle owl (*Bubo bubo*) to assess lead exposure associated to an abandoned mining area. Ecotoxicology 20:131–138

Griminger P, Scanes CG (1986) Protein metabolism. In: Sturkie PD (ed) Avian Physiology, 4th edn. Springer, Berlin, pp 326–344

Harr KE (2006) Diagnostic value of biochemistry. In: Harrison GJ, Lightfood TL (eds) Clinical avian medicine, vol I. Spix Publishing, Inc, Palm Beach, pp 611–630

Harrison GL, Harrison LR (1986) Clinical avian medicine and surgery. WB Saunders, London

Hawkey CM, Samour JH (1988) The value of clinical hematology in exotic birds. In: Jacobson ER, Kollias GV Jr (eds) Contemporary Issues in Small Animal Practice. Churchill Livingstone, London, pp 109–142

Hochleithner M (1994) Biochemistries. In: Ritchie BW, Harrison GJ, Harrison LR (eds) Avian medicine: Principles and application. Wingers Publishing, Lake Worth, pp 223–245

Hoefle U, Blanco JM, Sauer-Guerth H, Wink M (2000) Molecular sex determination in Spanish imperial eagle (*Aquila adalberti*) nestlings and sex related variation in morphometric parameters. In: Remple JD, Redig PT, Lierz M, Cooper JE, Lumeij JT (eds) Raptor Biomedicine III. Zoological Education Network, Inc., Lake Worth, pp 289–293

Jennings IV (1996) Appendix 8.1 haematology and clinical chemistry values. In: Beynon PH, Forbes NA, Harcourt-Brown NH (eds) Manual of raptors, pigeons and waterfowl. British Small Animal Vet Assoc Ltd, Cheltenham, pp 75–78

Johnson BL, Henny CJ, Kaiser JL, Davis JW, Schulz EP (2009) Assessment of contaminant exposure and effects on Ospreys nesting

along the Lower Duwamish River, Washington, 2006–07: U.S Geological Survey Open-File Report 2009-1255, 88 p

Johnston MS, Ivey ES (2002) Parathyroid and ultimobranchial glands: calcium metabolism in birds. Semin Avian Exotic Pet Med 11:84–93

Joseph V (1999) Raptor haematology and chemistry evaluation. Vet Clin N Am Exotique Anim Pract 2:689–699

Lanzarot MP, Montesinos A, San Andrés MI (2001) Hematological, protein electrophoresis and cholinesterase values of free-living nestling peregrine falcons in Spain. J Wildl Dis 37(1):172–177

Marchesi L, Sergio F, Pedrini P (2002) Costs and benefits of breeding in human-altered landscapes for the eagle owl *Bubo bubo*. Ibis 144: 164–177

Martínez JE, Calvo JF (2006) Búho real *Bubo bubo* (LINNAEUS, 1758). In: Robledano F, Calvo JF, Aledo E (eds) Libro Rojo de los Vertebrados de la Región de Murcia, Comunidad Autónoma de la Región de Murcia. Consejería de Industria y Medio Ambiente, Dirección General del Medio Natural, Región de Murcia, Murcia, pp 182–183

Martínez JA, Zuberogoitia I, Alonso R (2002) Rapaces Nocturnas. Guía para la determinación de la edad y el sexo en las estrigiformes ibéricas. Monticola Ediciones, Madrid

Martínez JA, Martínez JE, Mañosa S, Zuberogoitia I, Calvo JF (2006) How to manage human-induced mortality in the eagle owl *Bubo bubo*. Bird Conserv Int 16:265–278

Martínez-López E, María-Mojica P, Martínez JE, Peñalver J, Pulido M, Calvo JF, García-Fernández AJ (2004) Lead in feathers and δ-aminolevulinic acid dehydratase activity in three raptor species from an unpolluted mediterranean forest (Southeastern Spain). Arch Environ Contam Toxicol 47:270–275

Mateo R, Beyer WN, Spann JW, Hoffman DJ, Ramis A (2003) Relationship between oxidative stress, pathology, and behavioral signs of lead poisoning in mallards. J Toxicol Environ Health 66A:1371–1389

Mealey B, Parks GM, Pages CM, Millsap BA, Bass OL, Bossart GD (2004) Serum chemistry values for nestling bald eagles (*Haliaeetus leucocephalus*) in Florida Bay, Everglades National Park. J Raptor Res 38:96–100

Melling T, Dudley S, Doherty P (2008) The eagle owl in Britain. Brit Birds 101:478–490

Molina-López RA, Casal J, Darwich L (2011) Causes of morbidity in wild raptor populations admitted at a wildlife rehabilitation centre in Spain from 1995-2007: a long term retrospective study. PLoS ONE 6(9), e24603. doi:10.1371/journal.pone.0024

Monks DJ, Forbes NA (2007) Hematological. In: Zimmerman A, Bird DM, Bildstein KL, Barber DR (eds) Raptor research and management techniques. Hancock House Publishers, Canada, pp 278–285

Muriel R, Schmidt D, Calabuig CP, Patino-Martinez J, Ferrer M (2013) Factors affecting plasma biochemistry parameters and physical condition of Osprey (*Pandion haliaetus*) nestlings. J Ornithol 154: 619–632

Nakagawa S, Cuthill IC (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev 82: 591–601

Olsson O (2006) Genetic origin and success of reintroduced white storks. Conserv Biol 21:1196–1206

Penteriani V, Delgado MM, Campioni L, Lourenço R (2010) Moonlight makes owls more chatty. PLoS ONE 5(1), e8696. doi:10.1371/journal.pone.0008696

Pérez-García JM, Sánchez-Zapata JA (2015) Búho real (*Bubo bubo*). In: López-Iborra G et al (eds) Atlas predictivo de las aves nidificantes en la provincia de Alicante. Servicio de publicaciones de la Universidad de Alicante, España, pp 254–257

Pérez-García JM, Sánchez-Zapata JA, Botella F (2011) Distribution and breeding performance of a high-density eagle owl *Bubo bubo* population in southeast Spain. Bird Stud 59:22–28

Polo FJ, Celdrán JF, Peinado VI, Viscor G, Palomeque J (1992) Hematological values for four species of birds of prey. Condor 94: 1007–1013

Polo FJ, Celdran J, Viscor G, Palomeque J (1994) Blood chemistry of captive herons, egrets, spoonbill, ibis and gallinule. Comp Biochem Physiol 107A(2):343–347

Provencher Bolliger A, Everds NE, Zimmerman KL, Moore DM, Smith SA, Barnhart KF (2010) Hematology of laboratory animals. In: Weiss DJ, Wardrop KJ (eds) Schalm's veterinary hematology, 6th edn. Blackwell Publishing Ltd, Iowa, pp 852–887

Puerta ML, García del Campo AL, Abelenda M, Fernández A, Huecas V, Nava MP (1992) Hematological trends in flamingos, *Phoenicopterus ruber*. Comp Biochem Physiol 102A:683–686

Rosenthal R (1991) Metaanalytic procedures for social research, 2nd edn. Sage, Newbury Park

Schaub M, Pradel R, Lebreton JD (2004) Is the reintroduced white stork (*Ciconia ciconia*) population in Switzerland self-sustainable? Biol Conserv 119:105–114

Simkiss K (1967) Calcium metabolism in the laying bird. In: Simkiss K (ed) Calcium in reproductive physiology: a comparative study of vertebrates. Reinhold Publishing, New York, pp 155–197

Singer MA (2003) Dietary protein-induced changes in excretory function: a general animal design feature. Comp Biochem Physiol 136(B):785–801

Sonne C, Bustnes JO, Herzke D, Jaspers VL, Covaci A, Eulaers I, Halley DJ, Moum T, Ballesteros M, Eens M, Ims RA, Hanssen SA, Erikstad KE, Johnsen TV, Rigét FF, Jensen AL, Kjelgaard-Hansen M (2012) Blood plasma clinical–chemical parameters as biomarker endpoints for organohalogen contaminant exposure in Norwegian raptor nestlings. Ecotoxicol Environ Saf 80:76–83

Spagnolo V, Crippa V, Marzia A, Alberti I, Sartorelli P (2008) Hematologic, biochemical, and protein electrophoretic values in captive Tawny owls (*Strix aluco*). Vet Clin Pathol 37:225–228

Stout JD, Brinker DF, Driscoll CP, Davison S, Murphy LA (2010) Serum biochemistry values, plasma mineral levels, and whole blood heavy metal measurements in wild Northern goshawks (*Accipiter gentilis*). J Zoo Wildl 41:649–655

Thaxton JP, Puvadolpirod S (2000) Model of physiological stress in chickens 5. Quantitative evaluation. Poult Sci 79:391–395

Van Wyk E, van der Bank H, Verdoorn GH (1998) Dynamics of haematology and blood biochemistry in free-living African whitebacked vulture (*Pseudogyps africanus*) nestlings. Comp Biochem Physiol 120A:495–508

Villegas A, Sánchez JM, Costillo E, Corbacho C (2002) Blood chemistry and haematocrit of the Black vulture (*Aegypius monachus*). Comp Biochem Physiol 132A:489–497

Viñuela J, Ferrer M, Recio F (1991) Age-related variations in plasma levels of alkaline phosphatase, calcium and inorganic phosphorus in chicks of two species of raptors. Comp Biochem Physiol 99(A): 49–54

Warhol T (2007) Owls. Marshall Cavendish Benchmark, New York

Weick F (2006) Owls: strigiformes. Annotated and illustrated checklist. Springer, Berlin

Wolf SH, Schreiber RW, Kahana L, Torres JJ (1985) Seasonal, sexual and age-related variation in the blood composition of the Brown Pelican (*Pelecanus occidentalis*). Comp Biochem Physiol 82A:837–846