

# Age and sex-related differences in the haematological parameters of captive African grey parrots (*Psittacus erithacus*)

HELENA GASPAR<sup>1</sup>, FERRAN BARGALLO<sup>2</sup>, JORDI GRIFOLS<sup>2</sup>, ELISETE CORREIA<sup>3</sup>,  
MARIA DE LURDES PINTO<sup>4\*</sup>

<sup>1</sup>Animal and Veterinary Research Centre (CECAV), Trás-os-Montes e Alto Douro University, Quinta de Prados, Vila Real, Portugal

<sup>2</sup>Zoològic Veterinària, Badalona, Barcelona, Spain

<sup>3</sup>Mathematics Department, Trás-os-Montes e Alto Douro University, Quinta de Prados, Vila Real, Portugal

<sup>4</sup>CEMAT/IST-ID, Center for Computational and Stochastic Mathematics, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal

\*Corresponding author: [lpinto@utad.pt](mailto:lpinto@utad.pt)

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**Abstract:** African grey parrots (*Psittacus erithacus*) are very popular pets, commonly seen in avian clinical practice. Haematological profiles are critical to the understanding of several disease processes, being particularly useful as diagnostic tools in clinical practice, since birds tend to hide clinical signs of disease. We have previously proposed new haematological reference intervals (RI) for captive African grey parrots, and in the present work the basic data obtained was studied in detail to investigate the influence of factors, such as age and sex, on the haematological profile of this bird species. During an 8-year period (March 2009 to July 2017), animals ( $n = 239$ ) examined in first consultations or check-ups at the Zoològic Veterinària (Barcelona) were submitted to blood collection at different time points, rendering a total of 459 blood samples. The haematological testing was performed according to the guidelines of the American Society of Veterinary Clinical Pathology to determine the packed cell volume (PCV), haemoglobin (Hb), mean haemoglobin concentration (MHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total erythrocyte count (TRBC), total leukocyte count (TWBC), and differential leukogram with absolute and relative counts. All the haematological testing was performed in an in-house laboratory as previously described. Animals with 0 to 4 years of age showed higher values of PCV ( $P < 0.001$ ), Hb ( $P = 0.023$ ) and RBC ( $P = 0.018$ ), and lower values of MCHC ( $P = 0.008$ ), WBC ( $P = 0.012$ ) and heterophils ( $P < 0.001$ ) than older animals. There were significant differences exhibited in the monocytes ( $P = 0.035$ ) between different age groups. Females presented higher PCV, Hb and RBC values ( $P < 0.001$ ) compared to males. Our results suggest that the age and sex influence the haematological parameters in a significant manner in African grey parrots and should be accounted for when assessing the health status of individuals from this species.

**Keywords:** avian medicine; blood cell count; exotic pets; physiology

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Over the past decade, cage and aviary birds have dramatically increased in popularity as pets. The family Psittacidae is among the class Aves, which contains 370 species of parrots in 85 genera (Roskov et al. 2017). Psittacine species, with bright colours and morphological characteristics which means that they are not closely related to other orders of birds (Coles 2005), are the subject of particular interest both from families and from bird collectors. The African grey parrot (*Psittacus erithacus*), the largest species of parrots in Africa, has a high life expectancy and an unparalleled ability to imitate human speech and inanimate objects (Tully 2009; BirdLife International 2018), which makes it one of the most popular avian pets in Europe, the United States and the Middle East (Tully 2009; BirdLife International 2018). This popularity has a downside, as African parrots are among the most traded of all birds listed on the appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Martin 2018a; Martin 2018b). Historically, the African grey parrot has been traded since the 1870s (Annorbah et al. 2016), and a recent study pointed out the role of social media as a means to increase the local and global parrots trade (Martin et al. 2018). As a consequence, the export quotas for this species are probably being exceeded in several countries (Martin 2018a; Martin 2018b). For example, in Ghana, it is estimated that the decline of this species over the last two decades exceeds 90% (Annorbah et al. 2016). As of 2016, this species has been up listed from Vulnerable to Endangered in the IUCN Red List and it is now suspected to be undergoing rapid declines over three generations (47 years) (BirdLife International 2018), due to the local and international pet trade combined with habitat loss by agricultural activities, grazing, and rural population pressure amongst other anthropogenic factors (Berkunsky et al. 2017; BirdLife International 2018). Besides the conservation issues, the pet trade also raises biosecurity problems and often poses welfare and health risks for the animals involved.

Avian haematology is currently considered an integral part of clinical laboratory diagnostics in avian medicine. Despite haematology testing not providing an aetiological diagnosis, it does, however, provide information that is vital to assess an individual's health status, to monitor the progression of diseases, to evaluate the response to therapy and

to provide a prognosis (Samour 2006). Clinical haematology profiles are useful diagnostic tools in clinical practice that are particularly important for birds because they tend to hide clinical signs of diseases (Davis et al. 2008; Han et al. 2016). Mammalian haematology has advanced considerably over the past decades, and although some progress was also achieved in avian haematology, the latter still needs carefully controlled studies and investigation. Compared to mammalian species, data from the literature regarding different avian species is either limited or incomplete (Latimer and Bienzie 2000). More importantly, the creation of haematology databases is needed to establish reference intervals for various avian species (Samour 2006). In a previous study, we analysed the haematological parameters of African grey parrots, in order to establish new reference intervals for captive animals of this bird species (Gaspar et al. 2021). However, generalisations based on studies from a single species should be carefully made in avian patients, as greater seasonal variability has been reported among certain species of wildlife, related to moulting, reproduction and food supply, to name a few. In addition, most reports of pet bird blood reference intervals do not state age or sex distinctions. Furthermore, most of these studies consist of physically mature subjects (Fudge 2000a). This leads to a lack of comprehensive paediatric haematology guidelines, which challenge the clinician, since anaemic states may occur in neonates and nestlings due to management and nutritional problems as well as to some infectious diseases (Fudge 2000a). On the other hand, sex-related differences in haematological reference values can be clinically significant and must be interpreted correctly to ensure the appropriate diagnosis.

According to most of the literature, the differences between sexes are not significant in bird species (Fourie and Hattinch 1983; Tell and Citino 1992; Nazifi and Vesal 2003; Haefele et al. 2005; Foldenauer et al. 2007; Kolesnikovas et al. 2012; Prioste et al. 2012; Dolka et al. 2014; Doussang et al. 2015; Han et al. 2016). Despite this, there are studies in avian species that did find significant differences between sexes (Hauptmanova et al. 2006; Schmidt et al. 2009; Jones et al. 2014; Parsons et al. 2015). Regarding the haematology of the African grey parrot (*Psittacus erithacus*), which is one of the most frequently encountered psittacine species in avian medicine, little has been reported concerning the

influence of ageing in the haemogram. The same is also applied when sex differences are concerned. To this end, and after determining new reference intervals for the species (Gaspar et al. 2021), the haematological parameters of healthy African grey parrots (*Psittacus erithacus*) were analysed in detail to assess the differences in the blood indicators related to the age and sex. To the best of our knowledge, there are no studies to date on these factors and their impact on the haematological parameters in African grey parrots.

## MATERIAL AND METHODS

The animals and blood sample collection, as well as the methods used for the haematological testing were previously described and detailed in the paper by Gaspar et al. (2021).

### Animals

The African grey parrots included in this work ( $n = 239$ ) were selected through inclusion and exclusion criteria, as recommended by the guidelines of the American Society of Veterinary Clinical Pathology (ASVCP) (Friedrichs et al. 2012). The animals were examined in the first consultations or check-ups between March 2009 and July 2017 at the Zoològic Veterinària (Barcelona) and information regarding the age and sex of the animals recorded. During the period under study, nearly half of the animals were tested twice, at different ages, rendering a total of 459 blood samples for haematological testing.

### Blood sample collection, blood film and haematological testing

The animals were carried in their carrier or cage into the hospitalisation area and gently restrained, forming an Elizabethan grip. When sedation was necessary, 1 mg/kg (intranasal administration) of midazolam (Midazolam Normon EFG<sup>®</sup>, 15 mg/3 ml; Normon S.A., Madrid, Spain) was administered. The birds were moved to the examination table, and laid down horizontally, while maintaining the same Elizabethan grip. Phlebotomy was performed mostly in the right jugular vein as previously de-

scribed (Best 2005; Chitty 2005; Clark et al. 2009; Tully 2009; Bellwood and Andrasik-Catton 2014; Campbell 2015a; Campbell 2015b; Doneley 2016). The physical examination, sedation and phlebotomy took approximately 15 to 20 min altogether. Blood was collected into heparin and ethylenediaminetetraacetic acid (EDTA) tubes. Blood films were made using the whole blood without an anticoagulant and air dried before staining. The blood film staining was performed with a quick stain (Jorvet Dip Quick Stain; Jorgensen Labs, Inc., Loveland, CO, USA). The staining procedure was adapted from Campbell (2015a), and Bellwood and Andrasik-Catton (2014). Depending on the blood film thickness or the quality of the staining at the end of the process and, if necessary, the slide was further stained. The haematological testing was usually made on the same day of the blood collection. In order to provide usable data, all the materials and methodology remained constant throughout the study period, and the operator carrying out the laboratory procedures was always the same, complying with the ASVCP guidelines regarding pre-analytical and analytical procedures (Friedrichs et al. 2012). All the haematological testing was performed in an in-house laboratory as described previously (Gaspar et al. 2021) and is summarised in the following paragraphs.

### Packed cell volume (PCV)

The protocol used to determine the packed cell volume (PCV) was adapted from Campbell (1994) and Samour (2006). A capillary tube with well-mixed anticoagulated blood (from the EDTA tube) was filled to  $\frac{3}{4}$  and one end of the tube was sealed with clay.

The tube was centrifuged in a microhaematocrit centrifuge (DGMH-24; Diagnostic Grifols, SA, Barcelona, Spain) and then properly aligned in the microhaematocrit reader (Gri-Cel, SA, Barcelona, Spain) for the PCV determination.

### Total cell count: Erythrocytes and leucocytes

The total red and white blood cell count was determined using a manual method adapted from Campbell (1994), Samour (2006) and Campbell (2015c). The dilution used was 1 : 200, with Natt

and Herrick's solution (Vetlab, Miami, FL, USA). After waiting for the blood and solution to mix, an improved Neubauer chamber haemocytometer (Brand®, Wertheim, Germany) was covered with a coverslip, and one side of the haemocytometer was filled with the blood and stain solution. The cells were allowed to settle and then they were observed under a light microscope (BA210E Trinocular; Motic®, Carlsbad, CA, USA). The red blood cells (RBC) within the four corners and central squares (each one consisting of 16 smaller squares) as well as the ones that overlapped the top and left border of the Neubauer haemocytometer were counted ( $10 \times$  objective,  $100 \times$  magnification); the obtained number was multiplied by 10 000 to reach the total number of RBC per microlitre (or cubic millimetre). The white blood cells (WBC) within the nine larger squares on the grid, as well as all the cells that overlap the top and left border were counted using the same objective. Then 10% was added to the obtained value and multiplied by 200 (Campbell 2015c):

$$\text{WBC}/\text{mm}^3 = (\text{total leucocytes in 9 squares} + 10\% \text{ of total WBC}) \times 200 \quad (1)$$

where:

WBC – white blood cells.

These counts were repeated on the second side of the haemocytometer, and the counts from both chambers averaged.

### Haemoglobin concentration (Hb)

A quantitative method was used in this study to determine the haemoglobin concentration. The haemoglobin was oxidised into cyanomethaemoglobin and then measured by spectrophotometry (Campbell 2010). Drabkin's colorimetric solution was the reagent used (Spinreact 2012). The spectrophotometer (M100; Boehringer Ingelheim, Ltd, Biberach, Germany) was adjusted to zero with a blank solution (distilled water) at 540 nm. The whole uncoagulated blood (20  $\mu\text{l}$ ) was pipetted into a cuvette with Drabkin's solution (Spinreact, Girona, Spain). To allow for the conversion, the solution was mixed and incubated at room temperature. The absorbance of the sample was read at 540 nm to determine the haemoglobin concentration.

### Red blood cell indices

After the haematological variables (PCV, erythrocyte count and haemoglobin concentration) were determined, the secondary haematological parameters were also obtained using the following formulas (Clark et al. 2009):

$$\text{MCV (fl)} = \frac{\text{PCV (l/l)}/\text{RBC count}}{(10^6/\mu\text{l}) \times 1\,000} \quad (2)$$

where:

MCV – mean corpuscular volume;

PCV – packed cell volume;

RBC – red blood cells.

$$\text{MCH (pg)} = \text{Hb (g/l)}/\text{RBC count (}10^6/\mu\text{l)} \quad (3)$$

where:

MCH – mean corpuscular haemoglobin;

Hb – haemoglobin concentration;

RBC – red blood cells.

$$\text{MCHC (g/l)} = \text{Hb (g/l)}/\text{PCV (l/l)} \quad (4)$$

where:

MCHC – mean corpuscular haemoglobin concentration;

Hb – haemoglobin concentration;

PCV – packed cell volume.

### White blood cell differential count

To determine the white blood differential count (Campbell 1994; Samour 2006; Bellwood and Andrasik-Catton 2014; Campbell 2015c) the blood film in the monolayer area was examined under a microscope (BA210E Trinocular, Motic®) with a  $100 \times$  immersion oil objective. The slide was scanned in a methodical grid pattern, in order not to count the same area twice. As a routine, 100 WBC were counted (if the total WBC was increased, 200 WBC would be counted to maintain accuracy).

The relative WBC count was obtained by using the following formula (if 200 cells were counted, the division was by 200) (Bellwood and Andrasik-Catton 2014; Campbell 2015c):

$$\text{Cell type (\%)} = \frac{\text{number of cell type}}{\text{observed}/100} \quad (5)$$

The absolute white blood cell count was determined using the following formula (if 200 cells were counted, the division was by 200):

$$\text{Cell type} \times 10^3/\mu\text{l} = \frac{[\text{relative } (\%)/100] \times}{\times \text{WBC count } (10^3/\mu\text{l})} \quad (6)$$

where:

WBC – white blood cells.

### Statistical analysis

All the statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program (IBM SPSS Statistics v24).

To determine the effect of the age and sex on the dependent variables a two-way multivariate analysis of variance (MANOVA) was conducted followed by univariate analyses of variances (ANOVA) and Tukey's post hoc test, when appropriate. The significance level was set at  $P \leq 0.05$ . The data concerning the age and the sex of the animals included in this study ( $n = 239$ ) are expressed as the mean and standard deviation ( $M \pm SD$ ) or in a percentage. The different parameters of the haemograms ( $n = 459$ ) are expressed as the mean and standard deviation.

## RESULTS

### Animals

Regarding the age and the sex of the sampled animals ( $n = 239$ ), the mean age was  $5.57 \pm 5.15$  years old. There were 101 males (42.3%), 92 females (38.5%), and information about the sex was missing in 46 individuals (19.2%). In the males, the average age was  $5.46 \pm 4.58$  and in the females  $5.20 \pm 4.88$  years old.

### Haemograms

In order to encompass different life stages, the animals were divided into 3 age groups: early age, aged between 0 and 4 years old; sexual maturity adulthood, with individuals aged between 4 and 10; late adulthood, with subjects over 10 years old (Table 1). According to these categories, the distribution was as follows, 228 (49.7%) haemograms belonged to individuals aged between 0 and 4 years old, 159 (34.6%) to individuals aged between 4 and 10, and 72 (15.7%) to individuals aged over 10 years old. Significant differences were found in the PCV ( $P < 0.001$ ), Hb ( $P = 0.023$ ) and RBC ( $P = 0.018$ ), between the first age group and the

Table 1. Mean (M) and standard deviation ( $\pm SD$ ) of the African grey parrots' haematological parameters according to the age groups

Parameter	0 to 4 years of age ( $n = 228$ )	4 to 10 years of age ( $n = 159$ )	Over 10 years of age ( $n = 72$ )
	M ( $\pm SD$ )	M ( $\pm SD$ )	M ( $\pm SD$ )
PCV (%)	48.7 ( $\pm 5.0$ )*	46.4 ( $\pm 4.5$ ) <sup>+</sup>	46.8 ( $\pm 4.4$ ) <sup>+</sup>
Hb (g/l)	128 ( $\pm 11$ )*	125 ( $\pm 11$ ) <sup>+</sup>	128 ( $\pm 9$ ) <sup>+</sup>
RBC $\times 10^6/\mu\text{l}$	2.8 ( $\pm 0.3$ )*	2.7 ( $\pm 0.2$ ) <sup>+</sup>	2.7 ( $\pm 0.2$ ) <sup>+</sup>
MCV (fl)	173.0 ( $\pm 14.1$ ) <sup>+</sup>	171.2 ( $\pm 14.7$ ) <sup>+</sup>	169.4 ( $\pm 13.3$ ) <sup>+</sup>
MCH (pg)	45.6 ( $\pm 4.3$ ) <sup>+</sup>	46.3 ( $\pm 4.3$ ) <sup>+</sup>	46.5 ( $\pm 4.5$ ) <sup>+</sup>
MCHC (g/l)	264 ( $\pm 18$ )*	271 ( $\pm 20$ ) <sup>+</sup>	275 ( $\pm 24$ ) <sup>+</sup>
WBC $\times 10^3/\mu\text{l}$	5.8 ( $\pm 2.0$ )*	6.2 ( $\pm 2.4$ ) <sup>+</sup>	6.3 ( $\pm 2.6$ ) <sup>+</sup>
Heterophils $\times 10^3/\mu\text{l}$	3.3 ( $\pm 1.7$ )*	4.2 ( $\pm 4.2$ ) <sup>+</sup>	4.4 ( $\pm 2.3$ ) <sup>+</sup>
Lymphocytes $\times 10^3/\mu\text{l}$	2.2 ( $\pm 1.0$ ) <sup>+</sup>	1.9 ( $\pm 0.7$ ) <sup>+</sup>	1.6 ( $\pm 1.8$ ) <sup>+</sup>
Monocytes $\times 10^3/\mu\text{l}$	0.1 ( $\pm 0.1$ ) <sup>+</sup>	0.1 ( $\pm 0.1$ ) <sup>+</sup>	0.1 ( $\pm 0.2$ ) <sup>+</sup>
Basophiles $\times 10^3/\mu\text{l}$	0.01 ( $\pm 0.03$ )	0.01 ( $\pm 0.03$ )	0.01 ( $\pm 0.03$ )
Eosinophils $\times 10^3/\mu\text{l}$	0.03 ( $\pm 0.05$ )	0.02 ( $\pm 0.04$ )	0.03 ( $\pm 0.06$ )

Hb = haemoglobin concentration; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; PCV = packed cell volume; RBC = red blood cells; WBC = white blood cells  
\*<sup>+</sup>Values with different symbols are statistically different

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other two groups, with all of these parameters presenting higher values in the younger age group (juveniles). In these parameters, no statistically relevant differences were found between the two older age groups (Table 1). The MCHC ( $P = 0.008$ ) and heterophils ( $P < 0.001$ ) were significantly increased in the first age group when compared to the other two groups, but no significant differences were observed between the older groups.

There were significant differences ( $P = 0.012$ ) in the WBC between subjects from the first age group and the two older groups, with the younger animals having lower values. The values of the monocytes showed significant differences ( $P = 0.035$ ) between the first age group and the second group, as well as between the second and third age groups. Overall, the values related to the red blood cell line seemed to decrease with age, while the white blood cell values increased.

Regarding the sex, and taking the three different age groups into account, there were no significant differences between the number of haemograms belonging to females or males in each age category. Two hundred and four (44.4%) haemograms belonged to the females, 188 (40.9%) to the males and 67 (14.6%) to individuals in which the information about the sex was missing. Highly significant differences ( $P < 0.001$ ) were observed between the males and females regarding the PCV, Hb and RBC, with the females showing higher values of these parameters (Table 2). Although the other values, such as the WBC, lymphocytes, basophiles and eosinophils seemed to be higher in the females, no statistically significant differences were found.

## DISCUSSION

Several factors contribute to fluctuations in the blood homeostasis that have an impact on the haematological parameters; physiological factors, such as age and sex, but also captivity, diet, reproductive season/sexual maturity, season of the year, migration, photoperiod (Clark et al. 2009) and diverse stressful factors (Davis et al. 2008). Despite recent advances, research on avian haematology is still incomplete for many bird species. In a previous study, we proposed new reference intervals for captive African grey parrots (Gaspar et al. 2021) and, in the present study, it was deemed relevant to address the effect of the age and sex on the haemato-

Table 2. Mean (M) and standard deviation ( $\pm$  SD) of the African grey parrots' haematological parameters in the female and male subjects

Parameter	Male ( $n = 188$ )	Female ( $n = 204$ )
	M ( $\pm$ SD)	M ( $\pm$ SD)
PCV (%)	46.7 ( $\pm$ 4.9)*	48.6 ( $\pm$ 4.6)*
Hb (g/l)	125 ( $\pm$ 11)*	129 ( $\pm$ 10)*
RBC $\times 10^6/\mu\text{l}$	2.7 ( $\pm$ 0.2)*	2.8 ( $\pm$ 0.2)*
MCV (fl)	172.3 ( $\pm$ 14.1)	171.4 ( $\pm$ 14.4)
MCH (pg)	46.2 ( $\pm$ 4.3)	45.8 ( $\pm$ 4.3)
MCHC (g/l)	268 ( $\pm$ 20)	267 ( $\pm$ 19)
WBC $\times 10^3/\mu\text{l}$	6.0 ( $\pm$ 2.2)	6.1 ( $\pm$ 2.4)
Heterophils $\times 10^3/\mu\text{l}$	3.7 ( $\pm$ 1.9)	3.7 ( $\pm$ 2.2)
Lymphocytes $\times 10^3/\mu\text{l}$	1.9 ( $\pm$ 0.8)	2.1 ( $\pm$ 1.0)
Monocytes $\times 10^3/\mu\text{l}$	0.1 ( $\pm$ 0.1)	0.1 ( $\pm$ 0.1)
Basophiles $\times 10^3/\mu\text{l}$	0.00 ( $\pm$ 0.03)	0.01 ( $\pm$ 0.03)
Eosinophils $\times 10^3/\mu\text{l}$	0.02 ( $\pm$ 0.04)	0.03 ( $\pm$ 0.04)

Hb = haemoglobin concentration; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; PCV = packed cell volume; RBC = red blood cells; WBC = white blood cells

\* $P < 0.001$

logical parameters of this bird species. The average age of the individuals under study was 5.57 years old, being fairly similar for males (5.45 years old) and females (5.16 years old), which is relevant because the African grey parrot reaches sexual maturity between the ages of 4 and 6 (Marion and Carpenter 2013). The number of male and female subjects was also similar, making the sample more consistent.

In this study, the red blood cell parameters like the PCV, Hb, RBC and MCV showed a statistically significant decrease with the age. The values were higher in the age group that included juveniles, in agreement to what other studies have reported in different psittacine species. The measured values of the packed cell volume (PCV) and haemoglobin (Hb) were shown to be lower in nestling birds, increasing from 30 days to 180 days of age, in eclectus parrots (*Eclectus roratus*) (Clubb et al. 1990), macaws (Clubb et al. 1991a) and cockatoos (Clubb et al. 1991b). Additional studies comparing nestlings to adult values in other avian species showed similar results regarding the haemoglobin (Hawkey et al. 1984; Howlett et al. 1998; Schmidt

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et al. 2007; Hernandez and Margalida 2010; Han et al. 2016; Montolio et al. 2017). Furthermore, studies that compared the RBC total values of three or more age groups (Howlett et al. 1998; Hernandez and Margalida 2010), showed that these values increased while the animals are nestling and immature and then decrease when reaching adulthood. However, there are also reports of RBC counts being lower in younger individuals in several species of Psittaciformes, such as eclectus parrots (*Eclectus roratus*) (Clubb et al. 1990), macaws (Clubb et al. 1991a), cockatoos (Clubb et al. 1991b), red-capped parrots (*Pionopsitta pileata*) (Schmidt et al. 2009), and others (Villegas et al. 2004; Schmidt et al. 2007; Dolka et al. 2014; Jones et al. 2014; Montolio et al. 2017), although most of these studies only comprise samples from juveniles. The packed cell volume (PCV) has also been shown to be lower in young animals in budgerigars (*Melopsittacus undulatus*) (Harper and Lowe 1998) as well as in other bird species (Kocan and Pitts 1976; Hawkey et al. 1984; Howlett et al. 1998; Villegas et al. 2004; Dujowich et al. 2005; Lanzarot et al. 2005; Han et al. 2016; Montolio et al. 2017). In accordance with our results regarding the MCV, the data from the literature suggest that it is higher in younger individuals, namely in macaws (Clubb et al. 1991a), eclectus parrots (Clubb et al. 1990), and other species (Lanzarot et al. 2005; Dolka et al. 2014). On the contrary, studies in budgerigars (*Melopsittacus undulatus*) (Harper and Lowe 1998), the Oriental white stork (*Ciconia boyciana*) (Han et al. 2016) and in the horned guan (*Oreophasis derbianus*) (Cornejo et al. 2014) showed that younger animals presented lower values of the MCV. Regarding the MCH, this parameter showed an increase with the age from younger animals to late adulthood individuals, which is supported by studies conducted in psittacines (Clubb et al. 1990; Clubb et al. 1991a; Clubb et al. 1991b). The mean corpuscular haemoglobin concentration (MCHC) also significantly increased with the age, as was previously described in cockatoos (Clubb et al. 1991b), eclectus parrots (*Eclectus roratus*) (Clubb et al. 1990) and macaws (Clubb et al. 1991a). The higher values of these parameters in young animals seem to be related to an increase in the metabolic rate and, thus, a higher need for an oxygen supply due to the overall growth and sexual maturation. In fact, most studies report an increase in the RBC related parameters due to rapid tissue and feather growing

(Kocan and Pitts 1976), the high consumption of carbohydrates and protein (Campbell 2015b), as well as to the overall development in the physiological states of young animals (Dujowich et al. 2005; Hernandez and Margalida 2010).

The increase in the total white blood cell count (TWBC) with the age observed in our study is comparable to other studies in several species (Dolka et al. 2014; Han et al. 2016), namely in eclectus parrots (Clubb et al. 1990), cockatoos (Clubb et al. 1991b) and in Chilean flamingos (*Phoenicopterus chilensis*) (Hawkey et al. 1984). Other authors reported an increase in this parameter up to a certain age, and then a decrease, in macaws (Clubb et al. 1991a) as well as in other species (Howlett et al. 1998; Dujowich et al. 2005; Schmidt et al. 2007; Hernandez and Margalida 2010). The heterophil values also showed an increase with the age, as previously reported in cockatoos (Clubb et al. 1991b), eclectus parrots (Clubb et al. 1990) and ring-necked pheasants (Schmidt et al. 2007). In the Spix's macaw (*Cyanopsitta spixii*) (Foldenauer et al. 2007) and in captive bald eagles (*Haliaeetus leucocephalus*) (Jones et al. 2014), older animals also had higher heterophil counts. The increase in the heterophil values with the age observed in the present study cannot be attributed to the stress of restraint and blood sampling (Davis 2005), since, for all the animals under study, the sampling occurred in far less than an hour. On the contrary, the lymphocyte values showed a decrease with the age, in agreement to what was reported in the Spix's macaw (Foldenauer et al. 2007), although several studies mention that the lymphocyte count increases up to a certain age, and then decreases, in macaws (Clubb et al. 1991a), California condors (*Gymnogyps californianus*) (Dujowich et al. 2005), wild bearded vultures (*Gypaetus barbatus*) (Hernandez and Margalida 2010) and other species (Howlett et al. 1998; Schmidt et al. 2007). Studies in eclectus parrots (Clubb et al. 1990) and in cockatoos (Clubb et al. 1991b) have shown that the lymphocyte count is lower in young animals and later increases. Despite some of the mentioned studies pointing to different lymphocyte profile counts with the age according to the species under study, it is fair to presume that as the lymphocytes are part of the acquired immune system and highly specific, their number increases with the age, as animals come into contact with various pathogens. However, this increase requires a high

metabolic cost due to the need of rapid cell proliferation (Lochmiller and Deerenberg 2000; Lee 2006), a response that may be limited and compromised in older animals, justifying the lower values observed in this and other studies.

Additionally, our results suggest that the African grey parrot is a predominantly heterophilic species, having a higher circulation value of heterophils when compared to the lymphocytes, which is in accordance with previous reports in Psittaciformes (Fudge 2000b; Marion and Carpenter 2013). Furthermore, the ratio of heterophils to lymphocytes, the H/L ratio, increased with the age. Higher heterophilic profiles and an increase in the H/L ratio are often related to environmental and physiological stress, as well as to infection (Merino et al. 1999; Vleck et al. 2000; Davis et al. 2008). However, a study by Masello et al. (2009) in nestling burrowing parrots (*Cyanoliseus patagonus*) revealed that nestlings, in a better body condition, increased the number of heterophils and a positive relationship between the H/L ratio and body condition was registered. It was suggested that these values were probably related to a favoured investment in a robust constitutive innate immunity, which results in a reduced risk of infection in this long-lived species. The hypothesis that bird species with a greater life-span, such as *P. erithacus*, have evolved higher levels of immune response, innate immunity in particular, in order to assure its own survival against several pathogens and to protect future opportunities to reproduce was suggested by Tieleman et al. (2005), by measuring the bacteria killing activity in twelve long-living tropical bird species. These studies, together with the fact that the costs of sustaining a constitutive innate immunity are regarded as low compared to the lymphocyte development, due to the lack of a diversification process and low rates of cell turnover, when an immune response is not necessary (Lochmiller and Deerenberg 2000; Norris and Evans 2000; Lee 2006), could help to explain the high heterophil and H/L ratio observed in the older African grey parrots under study.

Taken together, the studies suggest that the RBC, PCV, Hb, WBC, heterophils and lymphocytes vary with age in a non-linear way: increasing up to a certain age and then decreasing (Hernandez and Margalida 2010; Dolka et al. 2014). The physiological fluctuations described in these studies are similar to those obtained in the three phases of life

under study in the present work. Overall, the present results suggest that the red blood cell parameters decreased with the age and the white blood cell parameters increased with the age in *P. erithacus*. Despite the underlying causes of these age-related variations, it still remains, to a large extent, unclear if they are a reflection of the metabolic demand imposed by the rapid growth and onset of the sexual maturation of this bird species, which is met by higher red blood cell counts in young animals, combined with a long life-span, during which the WBC are needed to respond to inflammatory processes, trauma and other stressful conditions. In this study, the increase in the WBC in older animals is mainly due to the heterophil values, suggesting the reinforcement of a non-specific protection mechanism against a diversity of stress factors, revealing a strong commitment in self-maintenance from animals of this long-lived species. In order for this to be ascertained, further studies dealing with the manipulation of both the innate and acquired immune system need to be performed.

In this study, the great majority of haemograms belonged to sexed individuals and the number of haemograms per sex was similar. As mentioned before, studies dealing with sex influences on the haematological parameters in birds are rather limited and comprised of very small samples. Albeit scarce, studies have been performed in psittacine species, which have not shown sex-related differences in the haematological parameters, such as in Spix's macaws (*Cyanopsitta spixii*) (Foldenauer et al. 2007), rose-ringed parakeets (*Psittacula krameri*) (Nazifi and Vesal 2003), golden conures (*Guaruba guarouba*) (Prioste et al. 2012), Cuban amazon parrots (*Amazona leucocephala*) (Tell and Citino 1992), hyacinth macaws (*Anodorhynchus hyacinthinus*) (Kolesnikovas et al. 2012), and other species (Fourie and Hattinch 1983; Haefele et al. 2005; Dolka et al. 2014; Doussang et al. 2015; Han et al. 2016). However, some authors suggest that sex differences in the haematological parameters do exist and might be associated with the reproductive activity, sexual maturation or differences in the sampling between the groups (Cornejo et al. 2014). A study conducted in captive horned guans (*Oreophaps derbianus*) revealed that juvenile males had lower MCV values than juvenile females, which was most likely related to the early physiological changes toward reproductive activity in the females, since the diet and husbandry were

identical in both sexes (Cornejo et al. 2014). Despite this, it is expected for females to present lower levels of RBC related parameters than males since oestrogen inhibits erythropoiesis and androgen stimulates it (Hauptmanova et al. 2006; Schmidt et al. 2009; Jones et al. 2014; Parsons et al. 2015). Contrary to this, and according to our results, this seems not to be the case in African grey parrots, since the PCV, RBC and Hb were significantly higher in the females. In this particular matter, the age did not influence the results, since the number of sampled males and females in each age group was fairly similar. Ots et al. (1998) reported higher PCV values in female great tits (*Parus major*), which they suggested could be a consequence of intense physical work, although no explanation was given as to why females would have more physical work. It has been reported that females that were not in the reproductive season had higher values of the RBC-related parameters compared to those who were mating, incubating or in courtship (Gayathri and Hegde 1994; Kalmbach et al. 2004).

It should be noticed that other haemogram values showed higher results in the females, namely the WBC, lymphocytes, basophiles and eosinophils. Although these values did not have significant differences between the sexes, some studies have reported similar results, both in wild (Allgayer et al. 2009; Plischke et al. 2010) and captive birds (Vergneau-Grosset et al. 2016).

In our study, the H/L ratio was higher in the males, as reported for adult burrowing parrots (*Cyanoliseus patagonus*) in the wild (Plischke et al. 2010), suggesting an inherent difference between the sexes in the haematological characteristics and immunity. Caution must be taken however when comparing the data from wild and captive animals, as it was demonstrated that captivity affects the avian leucocyte counts significantly (Ewenson et al. 2001).

This is the first study to use such a high number of samples to investigate age and sex variability in African grey parrots, suggesting that differences in the haematological parameters related to these physiologic variables should be accounted for in avian medicine. The values and variations herein described for *P. erithacus* further illustrate the strong differences between the phylogenetic groups and highlight the need for further studies in this and other bird species, both in captive and wild specimens.

## Conflict of interest

The authors declare no conflict of interest.

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