Acta Scientiae Veterinariae, 2024. 52: 1948.

RESEARCH ARTICLE
Pub. 1948

ISSN 1679-9216

Healthy Captive Rose - ringed Parakeets (*Psittacula krameri*) - Hematological and Biochemical Values

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ABSTRACT

***Background*:** Rose - ringed parakeets are popular pet birds worldwide. This study aimed to determine hematological and biochemical reference intervals for healthy Rose - ringed parakeets to help in the monitoring and early detection of diseases, preserving the health of birds and humans.

***Materials, Methods & Results*:** Blood samples were collected from 58 healthy adult Rose - ringed parakeets (33 males and 25 females) aged 5 - 10 years. The birds' health was assessed based on health history, physical examinations, and fecal examinations. Hematological and plasma biochemical analyses were conducted on the collected samples. The blood samples were collected from jugular vein with heparinized syringes. Blood smears were made immediately after the blood collection, and the samples were then packaged and sent to the laboratory. Hematological and biochemical analyses were performed within a maximum of 12 and 24 h after collection, respectively. The following biochemical profiles were analyzed: renal (uric acid), hepatic (GGT and AST), proteinogram (total protein, albumin, and globulin), muscular (AST, CK, and LDH), lipidic (total cholesterol and triglycerides), and electrolytes (calcium and phosphorus). Shapiro - Wilk test was used for normality analysis. The reference ranges were calculated using the package reference intervals R. The difference between males and females was analyzed using the Student *t* - test or the Mann - Whitney U test. The reference intervals for hematological parameters were as follows: RBC: 1.91 - 3.64 × 10^6/μL; PCV: 41.19% - 53.98%; HGB: 7.9 - 16.1 g/dL; MCV: 128.4 - 210.68 fL; MCH: 24.85 - 56.15 pcg; MCHC: 19 - 31.62 g/dL; WBC: 1.0 - 8.0 × 10^3/μL; heterophils: 0.134 - 4.678 × 10^3/μL; lymphocytes: 0.368 - 3.213 × 10^3/μL; monocytes: 0.033 - 1.440 × 10^3/μL; eosinophils: 0 × 10^3/μL; basophils: 0 - 0.760 × 10^3/μL; and heterophil:lymphocyte ratio: 0.12 - 2.97. The reference intervals for biochemical parameters were as follows: uric acid: 1.51 - 15.33 mg/dL; albumin: 1.04 - 2.64 g/dL; AST: 62.99 - 485.76 U/L; calcium: 6.79 - 10.18 mg/dL; CK: 321 - 3,279.51 U/L; total cholesterol: 191.15 - 446.49 mg/dL; phosphorus: 2.67 - 8.05 mg/dL; GGT: 7.2 - 29.37 U/L; globulin: 1.12 - 2.05 g/dL; LDH: 213.78 - 768.6 U/L; total protein: 2.7 - 4.2 g/dL; total plasma protein: 3.28 - 5.1 g/dL; and triglycerides: 41.1 - 502.02 mg/dL. No significant difference was observed between males and females for any parameters, except for the relative monocyte count, which was higher in males.

***Discussion*:** The results obtained in this study were based on a large number of birds. On erythrogram, differences between erythrocytes and hemoglobin were noted when compared with studies involving smaller populations (n = 16 and n = 20). These differences may be attributed to factors, such as age, breeding status, seasonal variation, and environmental factors. The hematocrit values were like another report with Rose - ringed parakeets (n = 16). On leukogram, the heterophils were more numerous, followed by lymphocytes. Eosinophils were rarely found. In the renal profile, uric acid values of 1.51 - 15.33 mg/dL were found, with no previous data specifically about uric acid in Rose - ringed parakeets but are considered within normal in birds range values up to 10 mg/dL. Higher values may have been influenced by the commercial formulated diet. On hepatic profile, GGT values were higher to that previously reported, but AST values were similar. On muscular profile, CK values were high and may be explained by physical restraint and containment stress. The lipidic profile (cholesterol and triglycerides) was influenced by diet, workout, climate, and hormones. Calcium and phosphorus values were higher than other reports. Calcium may vary with reproductive status, season. Diet may vary the phosphorus values. The values established in this report contribute to the diagnosis, control, and monitoring of diseases in Rose - ringed parakeets.

Keywords: Ring - necked parakeet, psittacine, clinical pathology, avian, avian medicine, exotic pets.

DOI: 10.22456/1679-9216.133961

Received: 28 October 2023 Accepted: 20 March 2024 Published: 23 April 2024

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INTRODUCTION

The Rose - ringed parakeet (*Psittacula krameri*) belongs to the Psittaculidae family and is a native species of sub - Saharan Africa and southern Asia [1,10,24]. These birds have been introduced to some countries worldwide, such as Europe and South America, by humans [1]. The Rose - ringed parakeets are among the most popular pets [18] as they are sociable, intelligent, and can imitate sounds. Thus, they are often taken to veterinary consultation, and exams are often performed to check their health.

Hematological and biochemical tests are minimally invasive procedures used to assess the health status and physiological condition of animals. However, limited knowledge exists regarding specific normal parameters in each species, as well as subtle differences in animals of different ages, health, or reproductive conditions [23].

Studies reporting hematological and blood biochemical values for Rose - ringed parakeets are scarce [8,16,20,26], and these studies have used small sample sizes. For instance, a hematological report with 16 birds (10 males and 6 females) [20], a biochemical report with 20 free range birds (sex not specified) [8], a hepatic evaluation report with 30 birds (20 males and 10 females) [26], and a report evaluating the impact of a medicament in hemoglobin values in 20 birds (10 males and 10 females) [16].

This study aimed to determine reference ranges for hematological and plasma biochemical parameters in a captive population of clinically healthy adult Rose - ringed parakeets using a consistent population. This is the 1st report of hematological and biochemical reference intervals in a healthy captive population of Rose - ringed parakeets (*Psittacula krameri*) in Brazil.

MATERIAL AND METHODS

Local and animals

This study included 58 clinically healthy adult Rose - ringed parakeets, 33 males and 25 females, from the Exotic Animal Incubation and Breeding Laboratory, located at the Experimental Farm Canguiri of the Federal University of Paraná (UFPR), Pinhais, PR, Brazil (25°23'13.4"S 49°07'34.2"W). The laboratory is situated in a humid temperate region with mild summers and maximum temperatures of 22ºC (according to Köppen classification). The birds were provided with a commercially formulated diet (Bambito)[1] and water *ad libitum*. Additionally, they received fruits and vegetables 3 times a week and seed mix (canary grass, millet, and sunflower seeds) twice a week. The birds were housed in community aviaries (49 m^2), located inside a masonry shed. The aviaries had curtains that were opened during the day and closed at night, ensuring proper air renewal and thermal comfort of the birds.

All the included animals were captive - born and ranged in age from 5 to 10 years. Sex determination was performed through visual examination, as males of this species become sexually dimorphic after reaching 3 years of age [18,24]. The selection of animals was based on their health history (no signs of disease 2 weeks before and until collection day), normal physical examination findings, and negative coproparasitological test results.

Samples

Animals were physically restrained, and blood collection was performed from the right jugular vein, using a 1 mL syringe heparinized with bovine sodium heparin (Heptar®)[2] and 26 G needle (0.45 × 13 mm). The maximum volume collected was 1% of the live weight of the birds [4]. Blood smears were prepared immediately after collection, and blood samples were stored in Eppendorf tubes, refrigerated and sent to the Laboratory of Veterinary Clinical Pathology at the Federal University of Paraná. Blood collections were carried out in the morning, and analyses were performed on the same day. The interval between collection and the start of processing in the laboratory was approximately 3 h.

Hematological exams

The hematological parameters evaluated included the total red blood cell (RBC) count, packed cell volume (PCV), hemoglobin concentration, mean corpuscular volume (MCV= PCV/RBC), mean corpuscular hemoglobin (MCH = hemoglobin concentration/RBC), mean corpuscular hemoglobin concentration (MCHC = hemoglobin concentration/PCV), total white blood cell (WBC), and differential leukocyte count. RBC and WBC counts were performed using a Neubauer chamber, with blood samples diluted 1:100 in a solution with brilliant cresyl blue. The PCV was determined by the microhematocrit method. After determining the PCV, the measurement of total plasma protein was performed using an optical refractometer

with temperature calibration (Instrutherm®)[3] [25]. Hemoglobin concentration was semi - automatically measured using the cyanmethemoglobin technique (colorimetric), with a commercial kit (Bioclin®)[4], and the readings were taken in a spectrophotometer (BA - 88A, Mindray®)[5] after centrifugation. Blood smears were stained using the May Grünwald Giemsa Modified method. For the May Grünwald Giemsa Modified stain, 16 drops of Giemsa[6] stain were placed in a graduated conical tube and filled with distilled water up to 10 mL. The slide was covered in the May Grünwald[6] stain solution for 2 min. Distilled water was carefully added on top of the solution until a water mirror was formed. After 1 min, the May Grünwald stain was discarded, and Giemsa solution was added on the slide for 10 min. After that time, the slide was washed and let dry.

Differential count of leukocytes was made in 100 cells (using a 100× objective - oil immersion). The estimation of the number of thrombocytes and erythrocyte evaluation was performed manually on blood smears. The slides were read by a trained and qualified professional. Erythrocyte morphology was assessed by size, color, and nucleus.

Plasma was obtained by centrifugation of the sample. Biochemical analyses were performed using automatic spectrophotometry equipment (BS - 200, Mindray®)[5]. The following parameters were measured: gamma glutamyltransferase (GGT) [Modified SZASZ IFCC method]; aspartate aminotransferase (AST) [kinetic UV method]; uric acid [colorimetric enzymatic method UOD - PAP]; total protein [Biuret method]; albumin [bromocresol green method]; globulins [total protein minus albumin]; creatinine kinase (CK) [UV kinetic method]; AST and lactate dehydrogenase (LDH) [UV kinetic method]; total cholesterol [COD - PAP colorimetric enzymatic method]; triglycerides [colorimetric enzymatic method]; calcium [colorimetric endpoint method]; and phosphorus [UV endpoint method]. Commercial kits (Bioclin®)[4] were used for the biochemical tests, calibration, and quality control.

Statistical analysis

All the statistical analyses were performed using R version 3.6.3 (The R Project for Statistical Computing)7 [22]. Outliers were identified using Cook's distance in the "reference intervals" R package [9]. If outliers were detected, they were removed. The results are presented according to the sample size, normality, mean, and standard deviation for the complete sample (males and females together) as well as for each gender. Variables are presented as mean and standard deviation. Normality was verified using the Shapiro - Wilk test. Significant sex differences were assessed using a *t* - test for normally distributed data or the Mann-Whitney U test for non - normally distributed data. First, data were checked for normality using the Shapiro-Wilk test. For normally distributed data, parametric RIs with 95% coverage and 90% confidence intervals for the RI bounds were calculated using the "reference intervals" R package. For data that failed in the Shapiro-Wilk test of normality, nonparametric RIs with 95% coverage and bootstrapped 90% confidence intervals for the RI bounds were calculated using the same package. For significant differences between males and females, RIs were calculated following the same procedure for each group. Differences in all analytic tests were considered significant at $P < 0.05$.

RESULTS

All 58 birds were considered healthy at the collect blood moment, based in their history, physical examination, and fecal exams and therefore were included in this study. Tables 1 and 2 summarize the reference ranges for hematological variables, whereas Tables 3 and 4 summarize the reference ranges for biochemical variables obtained.

On the erythrogram, reference interval for RBC count was determined as 1.91 - 3.64 × 10^6/μL, PCV as 41.19% - 53.98%, and hemoglobin as 7.9 - 16.1 g/dL. Mature psittacine erythrocytes are elliptical with orange - colored cytoplasm. The nucleus is central, elliptical, and purple in color [4]. Polychromatophils per field were estimated to be 1 and 6, in which the occurrence of polychromatophils in low numbers in healthy birds is considered normal [3]. Polychromatophils are similar in size to mature erythrocytes but may have a more rounded appearance. Their cytoplasm is slightly basophilic, and the nucleus is more rounded, and the chromatin is less condensed (Figure 1A). Metarubrocytes are smaller and spherical, with basophilic/grayish cytoplasm, depending on the stage of maturation (Figure 1B).

The differential leukocyte count revealed a predominance of heterophils (40.53%), followed by lymphocytes (40.24%). Eosinophils were rarely found. The mean heterophil:lymphocyte (H:L) ratio was 1.13 ± 0.72.

Table 1. Description of median, standard derivation for group, and sex-specific variation for hematological values and total plasma proteins for healthy Rose-ringed parakeets (*Psittacula krameri*) in the state of Paraná, Brazil

Hematological		Global		Female		Male	*P*-group
Parameter	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
RBC (10^6/µL)	56	2.77 ± 0.44	23	2.83 ± 0.43	33	2.73 ± 0.44	*0.43 (t)*
P-shapiro		*0.3*		*0.6*		*0.72*	
Hemoglobin (g/dL)	54	10.84 ± 1.89	23	10.98 ± 1.72	31	10.74 ± 2.03	*0.49 (mw)*
P-shapiro		*< 0.05*		*< 0.05*		*<0.05*	
PCV (%)	56	47.58 ± 3.26	25	47.92 ± 2.97	31	47.32 ± 3.50	*0.49 (t)*
P-shapiro		*0.19*		*0.31*		*0.23*	
MCV (fL)	54	169.53 ± 20.99	24	165.45 ± 20.49	30	172.8 ± 21.15	*0.20 (t)*
P-shapiro		*0.82*		*082*		*0.7*	
MCH (pcg)	56	39.92 ± 8.58	25	40.24 ± 8.84	31	39.67 ± 8.50	*0.81 (t)*
P-shapiro		*< 0.05*		*0.15*		*0.25*	
MCHC (g/dL)	54	23.01 ± 3.35	23	23.04 ± 3.30	31	23 ± 3.44	*0.91 (mw)*
P-shapiro		*< 0.05*		*< 0.05*		*< 0.05*	
Polychromatophils (/field)	55	3.72 ± 1.42	23	3.56 ± 1.34	32	3.84 ± 1.48	*0.47 (mw)*
P-shapiro		*< 0.05*		*0.15*		*< 0.05*	
WBC (10^3/µL)	56	3,875 ± 1,038,82	23	4,260.62 ± 2,055.31	33	3,606.06 ± 2,014.62	*0.29 (mw)*
P-shapiro		*< 0.05*		*0.09*		*< 0.05*	
Heterophils (%)	54	40.53 ± 10.70	24	41.54 ± 10.56	30	39.73 ± 10.92	*0.54 (t)*
P-shapiro		*0.18*		*0.13*		*0.34*	
Heterophils (10^3/µL)	57	1,637.36 ± 1,094.0	24	1,867.91 ± 1147.62	33	1,469.69 ± 1,038.75	*0.28 (mw)*
P-shapiro		*< 0.05*		*< 0.05*		*< 0.05*	
Lymphocytes (%)	54	40.24 ± 14.02	25	40.92 ± 11.87	29	39.65 ± 15.82	*0.73 (t)*
P-shapiro		*0.72*		*0.37*		*0.16*	
Lymphocytes (10^3/µL)	54	1,409.16 ± 802.87	22	1,636.59 ± 835.66	32	1,252.81 ± 753.03	*0.07 (mw)*
P-shapiro		*< 0.05*		*0.36*		*< 0.05*	
Monocytes (%)	55	10.83 ± 7.13	25	8.56 ± 6.47	30	12.73 ± 7.20	*0.01 (mw)*
P-shapiro		*< 0.05*		*< 0.05*		*0.07*	
Monocytes (10^3/µL)	54	417.40 ± 373.08	23	301.73 ± 187.89	31	503.22 ± 449.60	*0.33 (mw)*
P-shapiro		*< 0.05*		*0.34*		*< 0.05*	
Basophils (%)	56	6.16 ± 3.65	23	6.69 ± 3.84	33	5.78 ± 3.52	*0.37 (t)*
P-shapiro		*0.11*		*0.46*		*0.41*	
Basophils (10^3/µL)	55	230.63 ± 190.97	22	276.13 ± 230.33	33	200.30 ± 156.07	*0.28 (mw)*
P-shapiro		*< 0.05*		*< 0.05*		*< 0.05*	
H:L ratio	56	1.13 ± 0.72	25	1.19 ± 0.60	31	1.09 ± 0.81	*0.34 (mw)*
P-shapiro		*< 0.05*		*0.23*		*< 0.05*	
TPP (g/dL)	55	4.18 ± 0.46	24	4.12 ± 0.45	31	4.23 ± 0.47	*0.37 (t)*
P-shapiro		*0.09*		*0.32*		*0.18*	

RBC: red blood cells; PCV: packed cell volume; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; WBC: white blood cells. Data are presented as mean ± SD. P-Shapiro = *P*-value for the Shapiro-Wilk Normality Test. P-group = *P*-value for the Student *t*-test or the Mann-Whitney *U* test. Different superscripts (t, mw) are used to indicate if Student *t*-test or the Mann-Whitney *U* test was used, according to normality.

Heterophils are leukocytes characterized by a lobulated nucleus (2 to 3 lobes) with coarse and agglomerated chromatin. The cytoplasm is colorless and contains elongated (fusiform) granules that stain with an eosinophilic color (Figure 1C). Eosinophils have slightly eosinophilic cytoplasm, lobed nucleus, and granules are rounded and eosinophilic (Figure 1D). Basophils are round, slightly smaller than heterophils. The nucleus is generally not lobed, and the cytoplasm has round basophilic granules (Figure 1E). The lymphocytes are spherical and have a large nucleus - cytoplasm ratio, with clear to slightly basophilic homogeneous cytoplasm, and can be characterized as small, medium, and large (Figure 1F). Monocytes are large round cells. The nucleus varies in shape (round, oval, or lobed), with abundant blue - gray cytoplasm. They may have occasional vacuoles and fine eosinophilic granules (Figure 1G). To differentiate monocytes from large, reactive lymphocytes, the cytoplasm is evaluated. Monocytes have homogeneous blue - gray cytoplasm and are irregularly shaped, while reactive lymphocytes have basophilic cytoplasm, condensed chromatin, Golgi zone, and cytoplasmic vacuoles. Thrombocytes are small cells, may be oval or round, have dense nuclear chromatin, clear or pale blue - gray cytoplasm, may have 1 or more granules at the poles and vacuolation when activated (Figure 1H) [2,15,19].

No hemoparasites were found, and no morphological abnormalities were identified in erythrocytes or leukocytes. Total plasma protein values (refractometry) were 3.28 - 5.1 g/dL, while total protein reference value (Biuret) was 2.7 - 4.2 g/dL.

No significant differences were found in hematological and biochemical variables between males and females, except for the relative monocyte count ($P < 0.05$), which was higher in males than females, resulting in a reference interval for each gender.

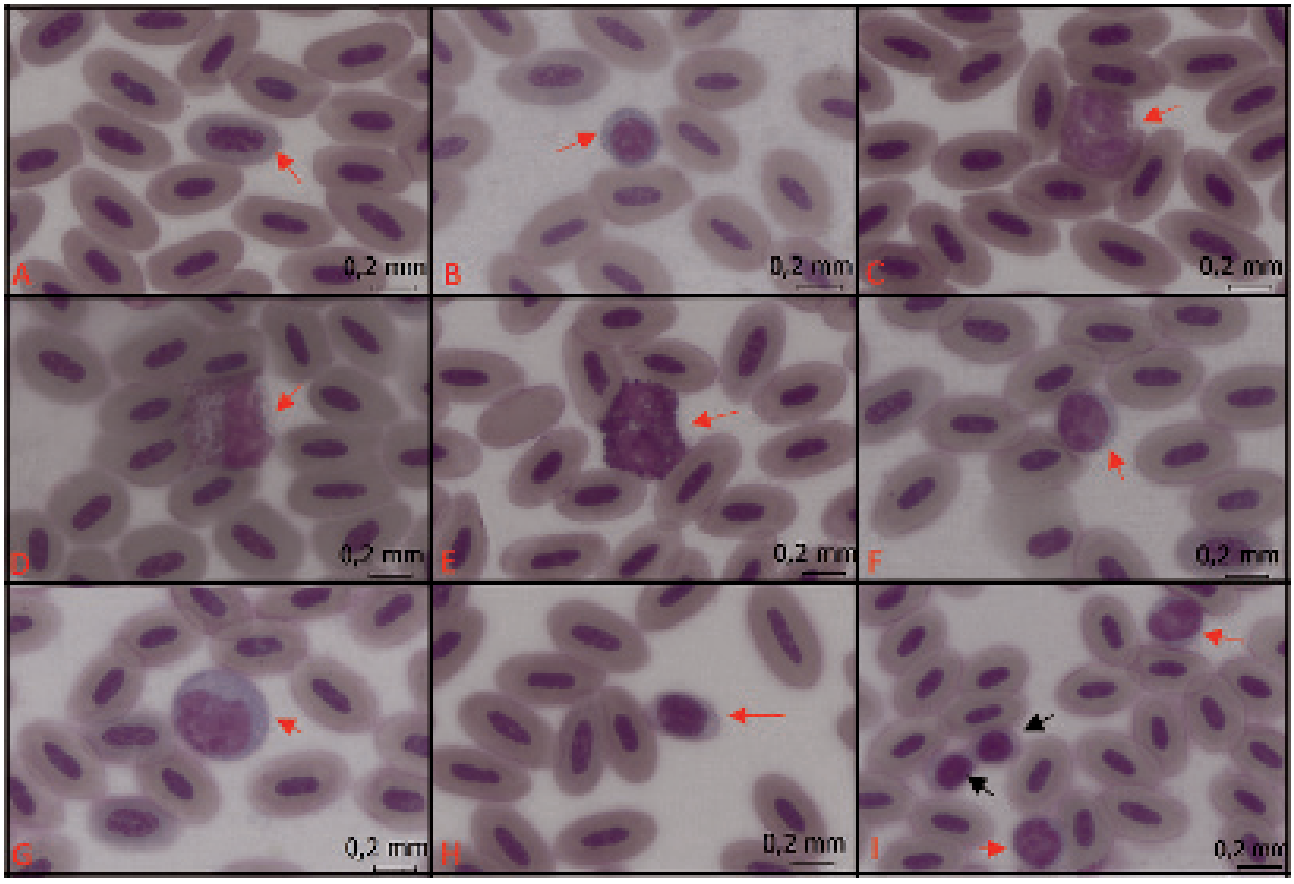

Figure 1. Peripheral blood of Rose - ringed parakeets (*Psittacula krameri*). A- Mature erythrocytes and a polychromatophil (arrow). B- Mature erythrocytes and a metarubrocyte (arrow). C- Heterophil (arrow). D- Eosinophil (arrow). E- Basophil (arrow). F- Lymphocyte (arrow). G- Monocyte (arrow). H- Thrombocyte (arrow). I- Two thrombocytes (black arrows) and 2 lymphocytes (red arrows). [May Grünwald stain solution (100×)].

Table 2. Reference interval, minimum and maximum values with 90% confidence intervals for hematological values and total plasma proteins for healthy Rose-ringed parakeets (*Psittacula krameri*) in the state of Paraná, Brazil

Hematological	Reference interval		Lower reference limit 90% CI		Upper reference limit 90% CI		Method
Parameter	Min	Max	Min	Max	Min	Max	
RBC (10^6/µL)	1.91	3.64	1.74	2.07	3.47	3.8	P
Hemoglobin (g/dL)	7.9	16.1	7.1	8.2	16.1	17.13	NP
PCV (%)	41.19	53.98	39.97	42.42	52.76	55.21	P
MCV (fL)	128.4	210.68	120.37	136.43	202.65	218.71	P
MCH (pcg)	24.85	56.15	21.7	25.7	55.3	57.3	NP
MCHC (g/dL)	19	31.62	19	19	31.25	33	NP
Polychromatophils (/field)	1	6	0	1	6	6	NP
WBC (10^3/µL)	1,000	8,000	1,000	1,000	8,000	8,850	NP
Heterophils (%)	19.56	61.52	15.46	23.65	57.42	65.61	P
Heterophils (10^3/µL)	134	4678	48	188	4516	5613	NP
Lymphocytes (%)	12.76	67.72	7.4	18.13	62.36	73.08	P
Lymphocytes (10^3/µL)	368.75	3,213.75	306.25	387.5	3,067.5	3,607.5	NP
Monocytes (%)	1	20	0	1	20	26	NP (F)
	0	26.84	0	2.31	23.15	30.54	P (M)
Monocytes (10^3/µL)	33.75	1440	12.5	37.5	1,440	1,620	NP
Basophils (%)	0	13.32	0	0.37	11.95	14.69	P
Basophils (10^3/µL)	0	760	0	0	720	950	NP
H:L ratio	0.12	2.97	0	0.14	2.95	3.24	NP
TPP (g/dL)	3.28	5.1	3.1	3.45	4.93	5.28	P

Min: minimum; Max: maximum; CI: confidence interval; P: parametric; NP: nonparametric; RBC: red blood cells; PCV: packed cell volume; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; WBC: white blood cells; (F) female; (M) male.

Table 3. Description of median. standard derivation for group, and sex-specific variation for plasma biochemical for healthy Rose-ringed parakeets (*Psittacula krameri*) in the state of Paraná, Brazil

Biochemical	Global		Female		Male		P-group
Parameter	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
Albumin (g/dL)	56	1.84 ± 0.40	24	1.77 ± 0.37	32	1.89 ± 0.42	*0.27 (t)*
P-shapiro		*0.24*		*0.23*		*0.39*	
AST (U/L)	56	213.90 ± 114.82	24	210.42 ± 118.05	32	216.52 ± 114.17	*0.73 (mw)*
P-shapiro		*< 0.05*		*< 0.05*		*< 0.05*	
Calcium (mg/dL)	54	8.48 ± 0.86	21	8.59 ± 0.96	33	8.41 ± 0.80	*0.49 (t)*
P-shapiro		*0.83*		*0.252*		*0.57*	
Cholesterol (mg/dL)	56	318.81 ± 65.13	25	302.02 ± 48.85	31	332.35 ± 73.79	*0.07 (t)*
P-shapiro		*0.88*		*0.17*		*0.71*	
CK (U/L)	54	1,255.34 ± 776.46	22	1,238.59 ± 779.40	32	1,266.59 ± 786.72	*0.99 (mw)*
P-shapiro		*< 0.05*		*< 0.05*		*< 0.05*	
GGT (U/L)	35	18.28 ± 5.65	14	16.60 ± 6.35	21	19.32 ± 5.05	*0.20 (t)*
P-shapiro		*0.23*		*0.24*		*0.59*	
Globulin (g/dL)	55	1.58 ± 0.23	24	1.59 ± 0.23	31	1.57 ± 0.23	*0.82 (t)*
P-shapiro		*0.08*		*0.12*		*0.22*	
LDH (U/L)	54	424.84 ± 141.79	24	447.08 ± 137.62	30	407.05 ± 144.86	*0.25 (mw)*
P-shapiro		*< 0.05*		*0.68*		*< 0.05*	
Phosphorus (mg/dL)	56	5.36 ± 1.37	24	5.47 ± 1.43	32	5.27 ± 1.34	*0.61 (t)*
P-shapiro		*0.94*		*0.95*		*0.42*	
Total Protein (g/dL)	57	3.38 ± 0.41	25	3.35 ± 0.41	32	3.40 ± 0.42	*0.56 (mw)*
P-shapiro		*< 0.05*		*< 0.05*		*0.3*	
TPP (g/dL)	55	4.18 ± 0.46	24	4.12 ± 0.45	31	4.23 ± 0.47	*0.37 (t)*
P-shapiro		*0.09*		*0.32*		*0.18*	
Triglycerides (mg/dL)	55	207.74 ± 122.71	23	204.10 ± 134.13	32	210.35 ± 115.95	*0.60 (mw)*
P-shapiro		*< 0.05*		*< 0.05*		*< 0.05*	
Uric acid (mg/dL)	56	7.54 ± 4.21	24	7.89 ± 4.56	32	7.28 ± 3.99	*0.44 (mw)*
P-shapiro		*< 0.05*		*< 0.05*		*< 0.05*	

TPP: total protein plasma. Data are presented as mean ± SD. P-Shapiro = *P*-value for the Shapiro-Wilk Normality Test. P-group = *P*-value for the Student *t*-test or the Mann-Whitney *U* test. Different superscripts (t, mw) are used to indicate if Student *t*-test or the Mann-Whitney *U* test was used, according to normality.

Table 4. Reference interval, minimum and maximum values with 90% confidence intervals for plasma biochemical for healthy Rose-ringed parakeets (*Psittacula krameri*) in the state of Paraná, Brazil

Biochemical	Reference interval		Lower reference limit 90% CI		Upper reference limit 90% CI		Method
Parameter	Min	Max	Min	Max	Min	Max	
Albumin (g/dL)	1.04	2.64	0.89	1.2	2.49	2.79	P
AST (U/L)	62.99	485.76	47.93	64.47	480.32	538.32	NP
Calcium (mg/dL)	6.79	10.18	6.46	7.12	9.85	10.51	P
Cholesterol (mg/dL)	191.15	446.49	166.68	215.62	422.02	470.96	P
CK (U/L)	321	3,279.51	188.78	353.4	3,165.92	3,620.72	NP
GGT (U/L)	7.2	29.37	4.47	9.93	26.64	32.1	P
Globulin (g/dL)	1.12	2.05	1.03	1.21	1.96	2.14	P
LDH (U/L)	213.78	768.6	184.11	218.35	748.2	849.03	NP
Phosphorus (mg/dL)	2.67	8.05	2.16	3.19	7.53	8.57	P
Total Protein (g/dL)	2.7	4.2	2.6	2.7	4.2	4.3	NP
TPP (g/dL)	3.28	5.1	3.1	3.45	4.93	5.28	P
Triglycerides (mg/dL)	41.1	502.02	5.3	51.9	495.54	548.82	NP
Uric acid (mg/dL)	1.51	15.33	0.94	1.73	14.57	16.76	NP

Min: minimum; Max: maximum; CI: confidence interval; P: parametric; NP: nonparametric; TPP: total protein plasma.

DISCUSSION

The present study reports hematological and plasma biochemical reference values for a healthy captive population of Rose - ringed parakeets. The sample size (58) was larger than previously reported studies. Since there are few studies reporting hematological and biochemical parameters in Rose - ringed parakeets, we have compared values of the present study with parameters obtained for other bird species of the same family, Psittaculidae, such as Orange - Bellied Parrot (*Neophema chrysogaster*) [11], when necessary.

The mean RBC count was 2.77×10^6/μL, which was lower than the value reported by in Rose - ringed parakeets in Shiraz, Iran (4.68×10^6/μL) [20]. This difference may have occurred because erythrocyte values can vary individually between birds of the same species. Other factors that can cause this variation in erythrocyte count are age, reproductive status, seasonal and geographic variation, hypoxia, and environmental factors [4,6].

Hemoglobin values (10.84 ± 1.89) were lower than those found in Rose - ringed parakeets in other two reports (13.20 ± 0.83 and 16.53 ± 0.53) [16, 20], but the determination techniques of hemoglobin were different between studies. PCV (47.58 ± 3.26%) values were comparable with those found in Rose - ringed parakeet in Shiraz, Iran (48.24 ± 1.24) [20]. The values of MCV (169.53 ± 20.99) were higher than those found in previous study with collared parakeet (103.51 ± 19.17) [20]. MCV values can increase with the age of the birds [4]. The birds in the present study were between 5 and 10 years old, whereas the birds in the other study were between 2 and 3 years old. The values of MCH (39.92 ± 8.58) were higher than in the collared parakeet (35.44 ± 6.98) [20]. The MCHC (23.01 ± 3.35) were lower than those previously found for the collared parakeet (34.29 ± 4.56) [20], but this MCHC reduction occurs due to the increase in MCV [4]. The mean WBC count was 3,875 ± 1,038.82 ($\times 10^3$/μL), close to values previously reported for Rose - ringed parakeet ($4.02 \pm 0.88 \times 10^3$/μL) [20]. Heterophils and lymphocytes were the most abundant leukocytes, with mean values of 40.53% (± 10.70%) and 40.24% (± 14.02%), respectively. Generally, heterophils are the most predominant leukocyte in the blood of birds [15]. In the present study, heterophils were slightly more abundant than lymphocytes, which is consistent with the findings in the Orange - Bellied Parrot [17]. The H:L ratio is used to assess physiological stress in birds and is particularly useful for assessing long - term environmental changes [11]. However, the health status of the birds must be considered, as inflammation can affect the H:L ratio. Additionally, morphological evaluation of leukocytes is also important [5]. The IR of H:L ratio in this study was 0.12 - 2.97. The relative basophil means values (6.16 ± 3.65) and relative monocytes mean values (10.83 ± 7.13) were higher than those reported in Shiraz, Iran (0.32 ± 0.36 and 2.63 ± 0.55, respectively) [20]. This difference found in the differential count of leukocytes among studies may have occurred due to the smaller number of animals in previous studies (n = 16). The established value for eosinophil count was zero. Few animals presented a single eosinophil.

The assessment of uric acid concentration is used to detect kidney disease in birds. Levels considered normal for uric acid in birds range from < 1 to 10 mg/dL [7]. The reference range determined for uric acid in the present study was 1.51 - 15.33 mg/dL (7.54 ± 4.21). Feeding of a commercial pelleted diet tends to increase uric acid levels [7], and this may explain the high RI for plasma uric acid levels since, in the present study, the birds receive pelleted food daily.

The GGT reference range in the present study was 7.2 - 29.37 U/L, higher than the range described to Indian Ring - necked parakeet (*Psittacula krameri manillensis*) (3 - 5 U/L) [26]. Heparin can react with GGT test reagents, causing turbidity, resulting in artificially elevated values [14]. Since we used heparinized syringes for blood collection, some samples may have contained more heparin than others, which may have caused this increase in the GGT interval. Differences in GGT values can also be caused by differences in methodologies used to measure enzyme [12].

The AST values in this study was 213.90 ± 114.82 U/L. AST is a very sensitive, but not very specific, indicator of hepatocellular disease and can be used to differentiate liver damage from muscle damage [13]. CK is a muscle - specific enzyme that can be utilized to detect muscle damage. In this study, the CK values ranged from 321 to 3,279.51 U/L, which were higher than those established for

Indian Ring - necked parakeet (682 - 981 U/L) [26]. Increased CK levels due to physical stress and exercise have been reported in healthy turkeys [14]. The high values of CK found in this study suggest that some stress occurred during capture and restraint for sample collection. During collection, the birds were removed from the aviaries with the aid of nets and placed in cages to be taken to the collection room. This handling may have contributed to the high CK values. LDH is present in several tissues of birds, but its increased activity is also commonly associated with liver disease and muscle damage. In healthy birds, LDH activity is considered normal up to 1,000 U/L [25]. The reference interval for LDH in the current study was 213.78 - 768.6 U/L.

The calcium intervals found in the present study were 6.79 - 10.18 mg/dL, which were higher than those found in Rose - ringed parakeet in Zaria, Nigeria (2.38 - 2.81 mg/dL) [8]. Calcium concentrations vary according to reproductive status, sex, and season [12]. The RI for phosphorus was 2.67 - 8.05 mg/dL, greater than Rose - ringed parakeet in another report (1.01 - 1.31 mg/dL) [8]. Phosphorus levels can be altered by diet. Diets with a predominance of seeds can lead to an increase in phosphorus [14]. In the present study, the birds received a mix of seeds 3 times a week *ad libitum*. The calcium and phosphorus levels can be used to evaluate the adequate intake of calcium and vitamin D3 and good mineral absorption [14].

Total protein can be used as an indicator of an animal's health status [14]. The reference value for total protein by the Biuret method in this study was 2.7 - 4.2 g/dL, which was lower than those found in Nigeria (6.53 - 7.7 g/dL) [8]. Total proteins can be influenced by age, hormones, diet, and temperature stress [14]. Plasma protein RI obtained by refractometry was 3.28 - 5.1 g/dL. This higher protein measurement in plasma is likely to be due to the presence of fibrinogen in plasma, but elevated glucose or cholesterol can also interfere with refractometer measurements of protein [25]. Albumin and globulin ranges were 1.04 - 2.64 g/dL and 1.12 - 2.05 g/dL, respectively, which were lower than those reported for Rose - ringed parakeet in Zaria, Nigeria (3.22 - 4.5 e 2.73 - 3.3 g/dL, respectively) [8]. Total protein, albumin, and globulin are used as a healthy indicator, and they can help in evaluating progression front a disease. However, changes in proteins levels can indicate dehydration or hyperhidratation [12,14].

The cholesterol reference interval was 191.15 - 446.49 mg/dL, greater than Indian Ring - necked parakeets (307 - 367 mg/dL) [26]. Higher cholesterol levels may result from a high - fat diet and poor exercise [21]. Triglycerides are the main form of lipid storage and an important source of energy [14]. The reference range for triglycerides in this study was 41.1 - 502.02 mg/dL. Several factors influence triglyceride levels, such as climate, hormones, diet, and gender [14]. In our study, there was no significant difference in plasma triglyceride levels between males and females.

CONCLUSION

Differences found between studies of *Psittacula krameri* in some parameters may be related to environmental and nutritional management, individual variation and the lower number of birds in previous studies. In this study, hematological and plasma biochemical reference ranges have been established for healthy adult captive Rose - ringed parakeets. The determined values contribute to aid in the diagnosis of diseases and to maintain health of these birds, preventing the spread of infectious, parasitic, and zoonotic diseases. This is the 1st report of hematological and biochemical reference intervals in healthy captive Rose - ringed parakeets (*Psittacula krameri*) in Brazil. This report was carried out with birds that were handled similarly to pet birds; therefore, to date, this is the most reliable reference value for clinical practice.

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***Ethical approval*.** This study was approved by the Animal Use Ethics Committee of the Agricultural Sciences sector of the Federal University of Paraná, Brazil (protocol 030/2021).

***Declaration of interest*.** The authors declare no conflicts of interest. The authors are solely responsible for the content and writing of the article.

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