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HEMATOLOGIC REFERENCE INTERVALS FOR THE ORANGE-WINGED AMAZON (*AMAZONA AMAZONICA*) KEPT AT A WILDLIFE MANAGEMENT ESTABLISHMENT IN THE AMAZON BIOME, PARÁ, BRAZIL[1]

INTERVALOS DE REFERÊNCIA HEMATOLÓGICOS DO PAPAGAIO CURICA (*AMAZONA AMAZONICA*) MANTIDOS EM ESTABELECIMENTO DE MANEJO DA FAUNA SILVESTRE NO BIOMA AMAZÔNIA, PARÁ, BRASIL

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ABSTRACT

Amazona amazonica, popularly known in Brazil as curica (Orange-winged Amazon), belongs to the order Psittaciformes and has a broad distribution across South America, occurring in all regions of Brazil. Although this species is frequently presented to veterinary care centers, hematologic data remain scarce, and hematologic reference intervals are essential to guide clinical interventions, monitor individuals under human care, and provide benchmarks for research. To establish hematologic reference intervals for the Orange-winged Amazon (*Amazona amazonica*) maintained at a wildlife management facility in the Amazon biome, state of Pará, Brazil. Blood samples were collected from 32 clinically healthy adult *A. amazonica*. Hematologic parameters were determined using manual and spectrophotometric methods. Reference intervals were computed with 90%

confidence, and outliers were excluded. Mean values for hemogram variables were as follows: erythrocytes, 2.42 million/µL; hemoglobin, 15.92 g/dL; hematocrit, 50.31%; MCV, 205.47 fL; MCH, 66.73 pg; MCHC, 31.7%; total leukocytes, 6,281.4 cells/µL; heterophils, 2,671.1 cells/µL; lymphocytes, 3,452.27 cells/µL; basophils, 22.63 cells/µL; monocytes, 106.41 cells/µL; thrombocytes, 29.44/µL; total plasma protein (TPP), 6.71 g/dL. Cellular morphological features were similar to those described for other psittacines. This study is the first to determine hematologic reference intervals for *A. amazonica* kept under managed care in the Amazon biome and may serve as a reference for conspecifics maintained under similar conditions.

Keywords: Conservation biology. Hematology. Avian medicine. Psittacines. Avian health.

RESUMO

Amazona amazonica conhecida popularmente como curica pertence à ordem dos psitaciformes e possui ampla distribuição geográfica na América do Sul, com ocorrência em todas as regiões do Brasil. Embora seja uma ave frequente em centros de atendimento veterinário, dados hematológicos da espécie são escassos e o intervalo hematológico é primaz para orientar intervenções clínicas, monitorar espécimes em ambientes sob cuidados humanos e fornecer referências em pesquisas. O objetivo do presente trabalho é estabelecer os intervalos de referência hematológicos do papagaio curica mantidos em estabelecimento de manejo de fauna no bioma Amazônia, estado do Pará, Brasil. Amostras de sangue foram coletadas de 32 papagaios A*mazona amazonica* adultos clinicamente saudáveis. Parâmetros hematológicos foram determinados por métodos manuais e espectrofotométricos. Os intervalos de referência foram calculados com 90% de confiança e outliers foram excluídos. Os valores médios encontrados das variáveis pesquisadas no hemograma foram: eritrócitos 2,42 milhões/µL; hemoglobina 15,92 g/dL; hematócrito 50,31%; VCM 205,47 fL; HCM 66,73 pg; CHCM 31,7%; leucócitos totais 6,281.4 células/µL; heterófilos 2,671.1 células/µL; linfócitos 3,452.27 células/µL; basófilos 22,63 células/µL; monócitos 106,41 células/µL; trombócitos 29,44/µL; PPT 6,71 g/dL. As características morfológicas das células mostraram-se semelhantes às de outros psitacídeos. Essa pesquisa é pioneira na determinação de intervalos de referências hematológicos de *Amazona amazonica* mantidos em mantenedouro no bioma Amazônia e pode ser utilizada como referencial a animais da espécie criados sob condições similares.

Palavras-chaves: Biologia da conservação. Hematologia. Medicina de aves. Psitacídeos. Saúde aviária.

INTRODUCTION

In Brazil, the avifauna comprises 102 families totaling 1,971 recognized species (Pacheco *et al*, 2021). Among them, the family Psittacidae stand out for their intelligence and sociability. This distinguishes them as common pets (Briscoe *et al*, 2010). Within this family, the genus *Amazona* is notable, with 12 species occurring in the country (Pacheco *et al*, 2021). One example is *Amazona amazonica* (Grespan; Raso, 2014), commonly known as the Orange-winged Amazon.

Despite its wide distribution, Orange-winged Amazon populations have been impacted by the destruction of natural habitats and illegal capture. This places them among the psittacines most often seized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) (Oliveira; Caparroz, 2007). Conversely, *Amazona amazonica* is classified as "Least Concern" on the IUCN Red List (IUCN, 2024). Even so, monitoring the health of these populations is necessary, given the impacts of human activities (Maceda-Veiga *et al*, 2015).

For such monitoring, peripheral blood is a principal indicator of an individual`s health. Hematologic analysis aids in disease diagnosis and supports population health surveillance. It also aids in assessing ecological integrity (Maceda-Veiga, *et al*, 2015). These tests screen for infectious, inflammatory, and metabolic diseases. They are essential in managing birds that are rescued and kept in breeding facilities (Clark; Boardman; Raidal, 2009).

A major challenge is the lack of reference values for avian species, both healthy and diseased, which limits how laboratory interpret results (Clark; Boardman; Raidal, 2009). Genetics, sex, age, physiologic state, territory, and habitat can all cause significant variation in hematologic variables (Samour, 2016). Some authors say it is inappropriate to use hematologic values from birds in different regions, even within the same species (Gomes *et al*, 2011).

Hematologic studies of *Amazona amazonica* under human care are scarce. Most available research comes from studies conducted in other countries (Cely; Ortíz, 2016; Vergneau-Grosset *et al*, 2016). Nationally we found only one publication (Cunha, 2021), a scientific abstract lacking information on sample size. Furthermore, have focused on birds from the Amazon biome. To address this gap, this study aims

to establish hematologic reference intervals for *Amazona amazonica* using individuals from a wildlife management facility in Pará, Northern Brazil.

MATERIALS AND METHODS

Ethical aspects

This study followed international animal welfare standards and was approved by the Animal Use Ethics Committee of the Federal University of Pará (UFPA) (protocol 4500260123). The SISBIO permit requirement was waived in accordance with Ordinance No. 748, issued on September 19, 2022, by the Chico Mendes Institute for Biodiversity Conservation (ICMBio), for *ex situ* collection of biological material.

Study area

The study was carried out at a wildlife management facility in Terra Alta, Pará, Amazon, Brazil (coordinates: 1.038° S, 47.908° W) (IBGE, 2010). The facility is located within the Amazon biome, which is known for its humid tropical rainforest climate.

Animal husbandry

The birds used in this study originated from seizures related to illegal wildlife trafficking and had lived at the facility for over five years under consistent environmental and dietary management. They were housed in screened group enclosures located in wooded areas that mimic features of their natural habitat. The enclosures allowed sun exposure at specific times of the day and maintained an average density of two birds per enclosure. Their diet consisted of previously selected and sanitized fruits—orange, açaí, papaya, guava, and banana.

Animal selection

The facility housed 36 adult *Amazona amazonica*. All birds underwent a physical examination to assess their overall health. The assessment included behavior, signs of dehydration, presence of ectoparasites, body condition score, dyspnea, traumatic lesions, and diseases such as poorly healed fractures, pododermatitis, feather loss, or neurological disorders. Based on these criteria and clinical history, the birds were considered clinically healthy.

All individuals were adults, and sex was not determined because the species is monomorphic, making it difficult to distinguish males from females based solely on morphological features (Doneley, 2018; Silva *et al*, 2020). Sampling was performed in

late March 2022, outside the reproductive season, which mainly occurs from August to December (Silva *et al*, 2020). The enclosures lacked suitable nesting substrates, preventing the start of the reproductive cycle and thus excluding reproductive influence on physiological parameters. Additionally, the absence of growing feathers and plumage defects indicated that the birds were not molting at the time of sampling.

Blood collection

Blood sampling followed the protocol described by Thrall (2015). Samples were collected in the morning between 07:00 and 08:00, before the first meal of the day, approximately 12 hours after the last meal. Birds were physically restrained, and the skin over the right or left jugular vein was aseptically prepared with 70% ethanol. Sampling was completed, on average, within 2 minutes of the researchers entering the enclosures. From each bird, 0.4 mL of whole blood was drawn using a 3-mL syringe and a 24G needle (0.55 × 20 mm) and immediately transferred to a tube containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). Samples were refrigerated for no more than 4 hours before laboratory analysis. Two blood smears per sample were prepared immediately after collection, using non-anticoagulated blood to avoid potential morphological and/or staining artifacts, and air-dried at room temperature.

Sample processing

Analyses were performed at the Veterinary Clinical Pathology Laboratory at the Federal University of Pará. Total counts of erythrocytes, leukocytes, and thrombocytes were manually determined using Natt–Herrick diluent (Êxodo Científica®) and a hemocytometer (Thrall, 2015). Hemoglobin concentration was measured spectrophotometrically with a portable analyzer (HemoCue® Hb 201+) (Gustavsson, 2015). Packed cell volume (PCV) was obtained by centrifuging whole blood in glass microhematocrit tubes for 5 minutes at 11,680 g (Benfer® Microhematocrit Centrifuge), and total plasma protein (TPP) was determined by refractometry (Thrall, 2015). The hematimetric indices—mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH)—were calculated as recommended in the literature (Thrall, 2015).

Blood smears were stained with May–Grünwald–Giemsa (Bioclin®) and **examined** under light microscopy for cellular morphology, differential leukocyte counts, and screening for hemoparasites at 1000× magnification.

Statistical analysis

Statistical analyses were performed in Microsoft® Excel using the Reference Value Advisor add-in (version 2.1), which adheres to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and the American Society for Veterinary Clinical Pathology (ASVCP) for establishing reference intervals in veterinary species.

First, descriptive statistics (mean, median, standard deviation, minimum, and maximum) were calculated. Data distribution was evaluated using the Anderson–Darling test, supported by frequency histograms and Q–Q plots to verify normality.

Lower and upper reference limits (RLs) were calculated using both standard and robust methods, with the Box-Cox transformation applied when the software automatically indicated it. Limits were established with 90% confidence intervals.

Outlier detection and removal were performed as needed using the Dixon–Reed and Tukey tests. Removing extreme values resulted in variations in sample size (n) across variables, depending on the criterion applied to each dataset.

RESULTS

In March 2022, blood samples were collected from 36 adult Orange-winged Amazons kept under human care at a wildlife management center in the Amazon biome, in the state of Pará, Brazil. All birds were considered clinically healthy. Of the samples collected, 32 met the criteria for laboratory analysis, while 4 showed clots and were excluded from the study.

Outliers were identified in specific individuals for MCV, total leukocytes, lymphocytes, heterophils, monocytes, basophils, and eosinophils. Accordingly, birds with results outside the normal range were excluded from the calculation of reference intervals. Mean values, standard deviations, medians, minimum and maximum values, as well as the reference intervals for each hematologic variable analyzed, are presented in Table 1.

Table 1: Hematologic reference intervals for Orange-winged Amazon (*Amazona amazonica*) from a wildlife management facility in the Amazon Biome, Pará, Brazil

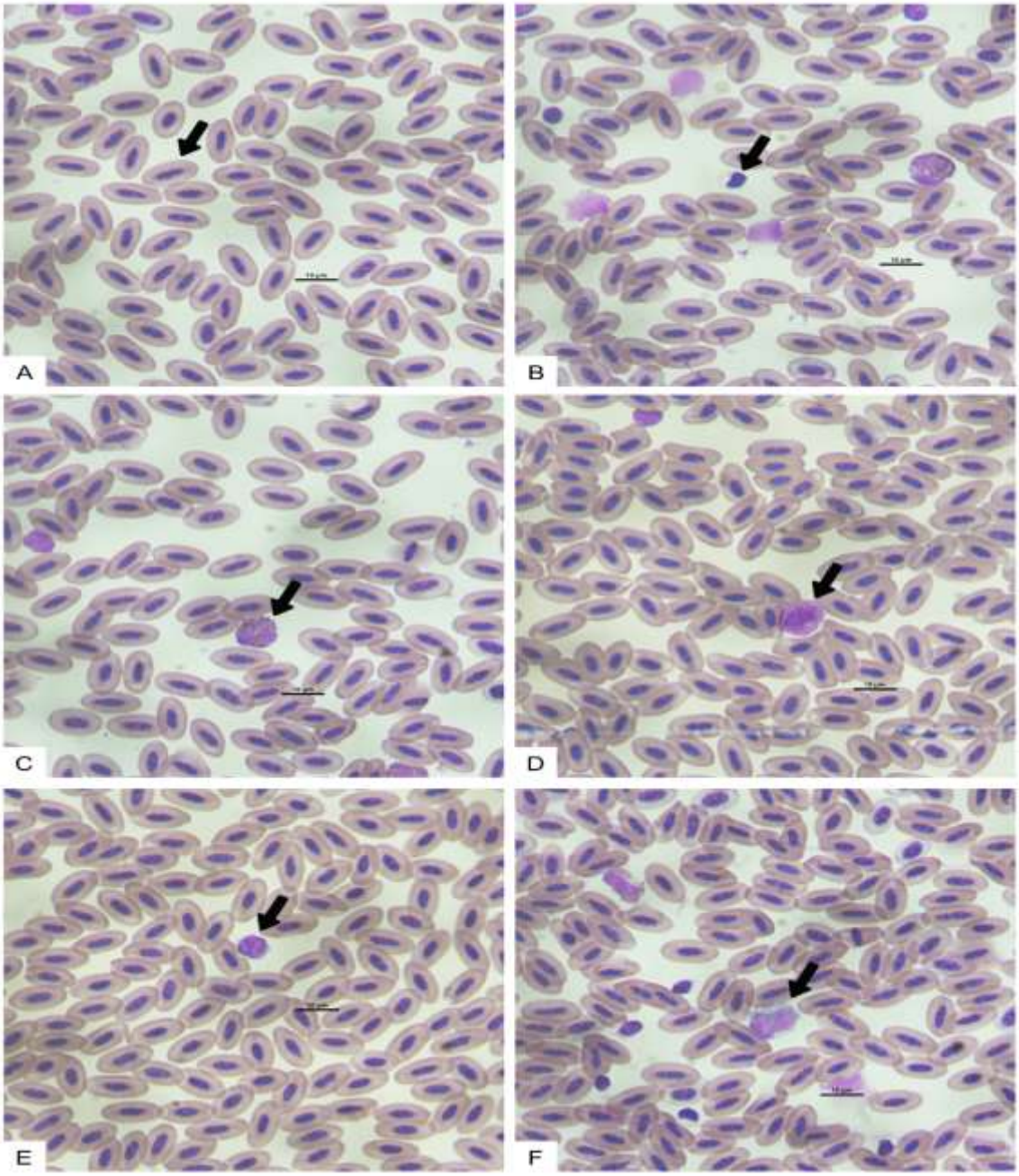
Variables	N	Mean	Median	Standard Deviation	Minimum	Maximum	Lower RL[1] (90% CI)	Upper RL[1] (90% CI)
Erythrocytes ($x10^6/mm^3$)	32	2.42	2.3	0.4	1.7	3.3	1.79 (1.69-1.89)	3.51 (3.02-4.14)
PVC (%)	32	50.31	50	3.81	44	62	43.6 (42.4-45.2)	58.9 (56.0-62.5)
Hemoglobin (g/dL)	32	15.92	16.2	1.55	12.1	18.6	11.86 (9.30-13.36)	18.51 (17.94-18.93)
MCV[2] (fL)	30	205.47	208.5	30.58	142.8	250	114.72 (38.55-151.07)	255.99 (245.49-265.89)
MCH[4] (pg)	32	66.73	69.95	12.62	48.4	91.1	40.81 (36.31-48.68)	95.41 (88.71-102.28)
MCHC[3] (%)	32	31.7	32.1	3.03	24.2	36.8	23.72 (18.55-27.02)	36.56 (35.60-37.36)
Thrombocytes/ µL	32	29.44	31	9.15	14	46	11,119.1 (8,189.3-16,394.9)	51,139.6 (45,617.8-55,449.4)
Total leukocytes/µL	29	6,281.4	6,160	1,304.97	3,960	8,800	3,879.36 (3,479.76-4,445.34)	9,456.28 (8,503.58-10,428.63)
Heterophils (%)	30	40	39	13	21	66	16 (ND -21)	71 (62 - 80)
Heterophils/µL	30	2,671.1	2,469.5	1,338.24	970	5,665	865.80 (708.95-1,115.28)	7,039.10 (5,443.99-9,008.60)
Band cells/µL	32	0	0	0	0	0	0 (ND[5])	0 (ND[5])
Eosinophils (%)	31	0	0	0	0	0	0.0 (00-0.0)	0.0 (0.0-0.0)
Eosinophils/µL	31	0	0	0	0	0	0.0 (0.0-0.0)	0.0 (0.0-0.0)
Basophils(%)	30	0,37	0	61	0	2	0.0 (0.0 - 0.0)	2,29 (0.95-ND[5])
Basophils/µL	30	22.63	0	35.82	0	92	0.0 (0.0 - 0.0)	85.3 (0.0999-ND[5])
Lymphocytes (%)	30	56	58	14	29	79	23 (13-35)	83 (77-88)
Lymphocytes/ µL	30	3,452.27	3,434	677.24	2,126	4,805	2,009.8 (1,677.95-2,467.21)	4,865.4 (4,532.49-5,176.73)
Monocytes(%)	29	1.6	1	1.4	0	5	0.0 (0.0 - 0.0)	5 (04-6)

Monocytes/µL	29	106.41	81	85.66	0	286	0.0 (0.0-1.96)	2,700.20 (1,150.02-4,315.44)
TPP (g/dL)	32	6.71	6.7	0.73	5.2	8.4	5.30 (5.00-5.62)	8.30 (7.87-8.71)

[1]RL — Reference Limit
[2]MCV — Mean Corpuscular Volume
[3]MCHC — Mean Corpuscular Hemoglobin Concentration
[4]MCH — Mean Corpuscular Hemoglobin
[5]Value not determined by the software
[6]TPP — Total Plasma Protein

Source: Clinical Pathology Laboratory, Federal University of Pará.

Figure 1: Blood smear morphology of Amazona amazonica: erythrocyte (A, arrow), thrombocyte (B, arrow), heterophil (C, arrow), basophil (D, arrow), lymphocyte (E, arrow), monocyte (F, arrow). May–Grünwald–Giemsa stain, 100× objective.



Source: Clinical Pathology Laboratory, Federal University of Pará.

No hemoparasites were found in any of the examined blood smears.

DISCUSSION

In veterinary clinical pathology, establishing reference intervals for wildlife species can be difficult. The main challenge lies in obtaining sufficient samples from animals, especially wild birds (Cray, 2015; Silva, Lima & Corrêa, 2020). In this study, 32 clinically healthy *Amazona amazonica* were analyzed. This sample size allowed the use of nonparametric statistical methods to estimate reference intervals, thereby improving the reliability of the results and minimizing the impact of outliers.

Studies aiming to establish reference intervals must consider multiple preanalytical and analytical variables that can affect avian hematologic values (Friedrichs *et al*, 2012; Montolio *et al*, 2017). In this study, only adult specimens were used, all on the same diet and sampled outside the reproductive and molting periods, resulting in a more consistent sample with fewer confounding factors. One limitation of our research is the absence of sex determination in curicas, as the species is not sexually dimorphic, making it impossible to distinguish sex solely by morphology (Sick, 1997).

Our literature review did not find studies reporting hematologic reference intervals for *A. amazonica* from wildlife management facilities within the Amazon biome. Therefore, this study takes a step toward addressing this specific topic in the referenced ecosystem, helping fill a gap in the scientific literature.

Regarding the erythron, the values obtained here differed from those reported for other *Amazona* species (Silva *et al*, 2016; Mello, 2016). Additionally, erythrocyte counts, PCV, and hemoglobin in our series differed from previous reports for *A. amazonica* in the United States and Colombia (Vergneau-Grosset *et al*, 2016; Cely & Ortíz, 2016). Such variation may reflect intrinsic factors (such as age, sex, reproductive status, and diet) as well as extrinsic factors (such as geography, climate, and analytical methodology) (Samour, 2016). For example, while Vergneau-Grosset *et al*. (2016) used automated counting for erythrocyte quantification, the present study employed manual techniques, which can contribute to inter-study variability.

Variations in hematimetric indices — MCV, MCH, and MCHC — have been reported among psittacines (Thrall, 2015). The MCV and MCHC values reported here for *A. amazonica* differ from those previously described (Polo *et al*, 1998) and from those reported for *A. rhodocorytha* (Mello *et al*, 2016) and *A. vinacea* (Silva *et al*, 2016). These differences reflect both individual and interspecific variation, since these indices are derived from erythrocyte count, hemoglobin, and hematocrit (Clark,

Boardman & Raidal, 2009), which themselves can vary with intrinsic and extrinsic factors (Campbell & Ellis, 2013).

Total thrombocyte counts showed significant variability in this study, especially at the lower and upper reference limits (11,119.1 to 51,139.6/µL, respectively). Notably, thrombocyte aggregates were seen on smears, which probably explain these fluctuations. Wide variation in thrombocyte counts has been reported in other species, such as *Amazona vinacea*, with values ranging from 17,000 to 116,000/µL (Silva *et al*, 2016). Despite these fluctuations, our results provide useful information on hematologic status; thrombocyte counts can be interpreted as decreased, adequate, or increased according to reference parameters (Clark, Boardman & Raidal, 2009).

In the leukogram, differences were observed in means, minima, maxima, and lower and upper reference limits for total leukocyte counts compared to studies of conspecifics (Cely & Ortíz, 2016; Vergneau-Grosset *et al*, 2016). These findings suggest that healthy individuals of the same species can display significant hematologic differences depending on their environment,as observed in the Andean–Amazon transition zone in Colombia (Cely & Ortíz, 2016), in the United States Vergneau-Grosset *et al*, 2016), and in the Brazilian Amazon. Additionally, values also differed from those reported for *Amazona vinacea* (Silva *et al*, 2016) and *Amazona aestiva xanthopteryx* (Sciabarrasi, 2019), underscoring that, despite belonging to the same genus, different species may present different total leukocyte counts. These results reinforce the importance of establishing species-specific reference values to avoid generalizations that could compromise diagnoses and clinical assessments.

Previous studies show that most psittacines have heterophils as the main leukocyte type, as seen in *Psittacula krameri* (Covaşă & Pavel, 2019) and *Psittacus erithacus* (Gaspar *et al*, 2021). However, species of the genus *Amazona* typically exhibit lymphocyte predominance (Doneley, 2011). This particularity was confirmed in the present study, where lymphocytes predominated over other leukocyte types—consistent with findings for *A. amazonica* in the United States (Vergneau-Grosset *et al*, 2016).

Heterophils were the second most common leukocyte in our samples, followed by monocytes, consistent with previous research (Vergneau-Grosset *et al*., 2016). Basophils ranked fourth, and eosinophils were not detected. This absence is expected physiologically, as eosinophils are usually sparse in avian blood smears (Pinto *et al*., 2016) and naturally circulate at low levels (Covaşă; Pavel, 2019), which explains their non-detection in the differential counts performed here. Similarly, no band cells were

observed, likely because the individuals were clinically healthy at the time of sampling, as confirmed by the pre-collection physical examination (Thrall, 2015).

Regarding leukocyte types and morphologic characteristics in *A. amazonica*, our findings are consistent with previously described cell features in other bird species and genera (Thrall, 2015). The identified cellular components resembled the morphotypes and morphological characteristics reported for other psittacines, as previously documented in *P. krameri* and *M. undulatus* (Covaşă & Pavel, 2019).

Total plasma protein concentrations varied significantly between the values obtained for *A. amazonica* in this study and those reported for other congeners, such as Amazona vinacea and *Amazona rhodocorytha* (Silva *et al*, 2016; Mello *et al*, 2016). This variation may be related to physiological factors such as age, basal metabolism, growth rate, and immune response (Vaz *et al*. 2015). Although reproductive status is also physiologically relevant, it did not apply to the individuals evaluated here. While pathological conditions can influence plasma protein levels, the birds assessed showed no clinical signs or histories suggesting illness.

Hemoparasite screening was conducted on blood smears, and no hemoparasites were detected, indicating the animals' good health. However, the method's inability to detect hemoparasites does not entirely rule out parasitic infection. Future studies should consider more sensitive techniques, such as molecular assays, to better detect potential hemoparasites. Therefore, despite the negative smear results, the presence of these agents cannot be excluded entirely.

CONCLUSION

This study is the first to establish hematologic reference intervals for the Orange-winged Amazon (*Amazona amazonica*) at a wildlife management facility within the Amazon biome, providing valuable parameters for clinical assessment, health management, and species conservation.

Furthermore, comparisons with other studies revealed physiological variations caused by climate, geography, and methodology, enhancing the need for region- and management-specific reference values. The population-specific features support this research and emphasize the importance of continued research in this field.

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