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Original Study

Hematological and Plasma Biochemical Reference Values for Captive White-Fronted Parrots (*Amazona albifrons*) in México

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Abstract: The objective of this study was to establish hematological and plasma biochemical reference values in captive white-fronted Amazon parrots (*Amazona albifrons*), as well as to determine whether sex effects the reference values. To our knowledge, hematological and plasma biochemical data have not been reported in this species. Thirty-seven clinically healthy adult individuals (21 males, 16 females) from El Nido Bird Sanctuary, Ixtapaluca, Estado de México, were the subject birds for this study. Complete blood count and selected plasma biochemical parameters, including uric acid, glutamate dehydrogenase, aspartate aminotransferase, total protein, and albumin, were evaluated. Blood samples were collected in the winter (January), outside of the birds' breeding season. Many hematological and plasma biochemical analytes had large coefficients of variation, and there were no statistically significant sex differences identified.

Key words: blood analytes, hematology, plasma biochemistry, sex, avian, white-fronted Amazon parrots, psittacine, *Amazona albifrons*

INTRODUCTION

The white-fronted parrot (*Amazona albifrons*) is a diurnal, gregarious, medium-sized psittacine (25.5–29 cm) that weighs between 176 and 242 g. This species is characterized by bright green plumage, a short tail, yellow beak, gray legs, amber eyes, and gray orbital rings. White-fronted parrots exhibit marked sexual dimorphism, with the primary covert feathers of the males being red and the white forehead being attenuated in females.[1] The range for this species extends from northwestern México to Costa Rica on low and medium slopes in rainforests below 1800 m (above mean sea level).[2]

Hematological and biochemistry testing is commonly used to assess the health of avian patients. However, for these results to have clinical value, it is important to have reference intervals from a controlled population for comparison. Following an extensive literature search, the authors could not find any published hematological or plasma biochemical reference values for adult captive *A albifrons*. Reference values for biochemical analytes of psittacine birds have been established for specific species of the genera *Psittacus*, *Amazona*, *Cacatua,* and *Ara*, and although there are published values for 6 different *Amazona* species, the reference values represent combined averages of all 6 species used for the entire genus *Amazona*.[3] Hematological and plasma biochemical values have been reported for 19 psittacine species, including parrots, cockatoos, macaws, conures,

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African gray parrots (*Psittacus erithacus*), and *Amazona* species, including the orange-winged Amazon parrot (*Amazona amazonica*, n = 5), turquoise-fronted Amazon parrot (*Amazona aestiva*, n = 7), festive Amazon parrot (*Amazona festiva*, n = 6), Cuban Amazon parrot (*Amazona leucocephala*, n = 7), yellow-crowned Amazon parrot (*Amazona ochrocephala*, n = 8), and vinaceous-breasted Amazon parrot (*Amazona vinacea*, n = 5). Plasma biochemistries were evaluated in only 6 of the 8 yellow-crowned Amazon parrots.[4]

In 2008, the hematological values of 56 *Amazona* parrots from 6 different species were evaluated. The majority of the parrots sampled in the study were yellow-shouldered Amazon parrots (*Amazona barbadensis*, n = 20) and yellow-crowned Amazon parrots (n = 16).[5] Hemogram and biochemical values have also been published for orange-winged Amazon parrots,[6] red-lored Amazon parrots (*Amazona autumnalis*),[7] vinaceous-breasted Amazon parrots,[8] and red-capped parrots (*Pionopsitta pileata*).[9] Some of these studies included small sample sizes.[3–5,7,9] Only 1 of these previously described studies evaluated the reference intervals for differences between the sexes, and that study of red-lored Amazons found no differences.[7] However, differences in hematological results have been documented in some species of birds, including the pileated parrot (*Pionopsitta pileata*),[9] horned guan (*Oreophasis derbianus*),[10] common pheasant (*Phasianus colchicus*),[11] Galapagos penguin (*Spheniscus mendiculus*),[12] and the burrowing parrot (*Cyanoliseus patagonus*).[13] Mute swans (*Cygnus olor*),[14] like the red-lored Amazons,[7] were also found to have no differences in hematological values between sexes. These findings suggest that species-specific reference intervals should consider the potential influence of sex on the results.

The white-fronted Amazon parrot is frequently represented in zoological collections and maintained as a companion animal. Therefore, established hematological and plasma biochemical reference values are of veterinary importance, and extrapolation of reference intervals between different populations should be done with caution. The objective of this study was to establish hematological and plasma biochemical reference intervals and to determine the effect of sex on the results in captive white-fronted parrots. We hypothesized that hematological and plasma biochemical values will differ between male and female white-fronted Amazon parrots.

MATERIALS AND METHODS

Bioethical committee

This study was evaluated and approved by the Internal Committee for Use and Animal Care (CICUA), Facultad de Medicina Veterinaria y Zootecnia de la Universidad Nacional Autónoma de México (folio number: 088).

Animals

Thirty-seven >6-months-old white-fronted parrots (21 males, 16 females) from the El Nido Bird Sanctuary, Ixtapaluca, Estado de México, were included in this study. At the time of the study, all the birds were housed in a 3 × 5 × 3 m mesh and concrete outdoor enclosure with a natural photoperiod. The birds' diet consisted of fruits, vegetables, sunflower seeds, and commercial Pedigree dog food (Mars, Inc, El Marques, Queretaro, México). The health of each bird was assessed by an external physical examination of the eyes, external nares, oral cavity, choana, mucous membranes, skin, plumage, body condition, and body weight. Birds exhibiting any type of disease or under medical treatment 30 days prior to the study were excluded.

Sample collection

Sample collection took place in January (winter) to avoid the breeding season of the birds. The night before blood collection, the birds' food was removed. All samples were obtained between 9:00 AM and 11:00 AM. Prior to blood collection, the area over the vein was disinfected with a 70% ethyl alcohol swab. Heparinized (5000 U/mL sodium heparin; Inherpar, Laboratorios PiSA, Jal, México) 1-mL syringes and 25-G needles were used for phlebotomy of the right jugular vein.[15] Animals were physically restrained as described for the species.[16] Once the blood was collected, blood smears were made and the remainder of the sample placed in a microtainer blood collection tube without additives (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

Sample preparation

Two blood smears were prepared immediately after specimen collection using the wedge technique with 25 × 75-mm slides, labeled, and stored in a slide holder for further processing. The remainder of the samples were placed in racks at room temperature and then refrigerated at 39.2°F (4°C) within 3 hours of collection. Samples were transported and processed at the Clinical Pathol-

ogy Laboratory of UNAM, Facultad de Medicina Veterinaria y Zootecnia.

Hematological analysis

Samples were mixed, immediately placed in 2 nonheparinized capillary tubes (Científica Velaquin, Mexico City, México), and centrifuged at 11,000 rpm for 5 minutes. The gross appearance (hemolysis, icteric, or lipemic) of the sample was recorded. Packed cell volume (PCV) was determined visually using a scaled hematocrit reader (Thermo Fisher Scientific, Waltham, MA, USA). The capillary tube plasma column, above the buffy coat, was examined for microfilariae by light microscopy at low magnification (×10).[17,18]

The 2 blood smears were stained with water/alcohol-soluble Wright stain (Hycel, Jal, México), air-dried, and mounted with resin and a coverslip.[17] The feathered edge of the blood smear was evaluated with a ×10 objective to screen for thrombocyte aggregation. The thrombocyte count was estimated in the blood smear at ×100. The estimate of 5 fields at ×100 was averaged and multiplied by 3.5 to obtain thrombocytes $\times 10^9$/L.[19] Samples with thrombocyte aggregates were excluded from the thrombocyte count.

Differential white blood cell counts were performed by a single board-certified pathologist using a ×100 objective and counting 100 cells per slide. Erythrocyte and leukocyte counts were determined with a hemocytometer using the Natt and Herrick solution and compared with slide estimates.[19] Total solids were determined with a refractometer (Hand Refractometer, BOECO, Hamburg, Germany).[18] Mean corpuscular volume (MCV) was estimated using the formula (PCV × 1000/number red blood cells).[18] Coagulated or low-volume samples were excluded for analysis.

Plasma biochemical analysis

Biochemistry testing was performed within 8 hours of blood collection. A spectrophotometer (Dirui CS-T240, Diagnostics Chemicals Limited, Charlottetown, Prince Edward Island, Canada) was used to determine uric acid, glutamate dehydrogenase (GDH), aspartate aminotransferase (AST), total protein, and albumin concentrations. Uric acid concentrations were measured with a buffered solution containing 1.8 mmol/L dichloro-2-hydroxybenzenesulfonic acid, 0.5 mmol/L 4-aminoantipyrine, >3500 U/L peroxidase (botanical), >200 U/L uricase (microbial), stabilizers, and preservatives (Sekisui Diagnostics, Charlottetown, Prince Edward Island, Canada). Glutamate dehydrogenase was measured using buffer/substrate (triethanolamine buffer 50 mmol/L, pH 8.0; ammonium acetate, 100 mmol/L; ethylenediaminetetraacetic acid, 2.5 mmol/L); enzyme/coenzyme (adenosine diphosphate 1.0 mmol/L, nicotinamide adenine dinucleotide 0.2 mmol/L; lethal dose ≥ 2 U/mL); and α-oxoglutarate (7 mmol/L, Sekisui Diagnostics). Aspartate aminotransferase was measured using an enzyme reagent solution containing a buffer (pH 7.8 at 77°F [25°C]), 240 mmol/L L-aspartate, >600 U/L malate dehydrogenase (microbial), >600 U/L lactate dehydrogenase (microbial), a preservative, and a substrate reagent solution containing 12 mmol/L 2-oxoglutarate, >0.18 mmol/L nicotinamide adenine dinucleotide, and a preservative (Sekisui Diagnostics). Total protein was measured with a solution containing 31.8 mmol/L sodium potassium tartrate, 12.0 mmol/L copper sulfate pentahydrate, 30.1 mmol/L potassium iodide, and 0.20 mol/L sodium hydroxide (Sekisui Diagnostics). Albumin was measured with a solution containing acetate buffer (pH 4.2 at 77°F), at least 0.39 mmol/L bromocresol green, a surfactant, and a preservative (Sekisui Diagnostics).

Due to the limited blood sample volumes, samples could not be analyzed in duplicate. To limit observer influence, all determinations were performed by a single, board-certified pathologist working in an ISO 9001:2015–certified clinical pathology laboratory for quality standards in specimen processing.

Statistical analysis

The hematological and biochemistry values were sorted by sex. Outliers were identified and eliminated using Tukey's method of interquartile fences.[20] A Kolmogorov-Smirnov test and Levene's test were used to evaluate the distribution (normality) and homogeneity of variance of the data, respectively.[20] Variables meeting the assumptions of a Gaussian distribution and homogeneity of variance were compared by parametric analysis (independent samples *t* test), whereas variables that did not meet these assumptions were compared with nonparametric methods (Mann-Whitney test). The minimum, maximum, mean, standard deviation, and coefficient of variation (CV) were determined for all hematological and biochemical data. Median values were also calculated for non-normally distributed data. Statistical analysis was performed using commercial statistical software (SPSS version 28; IBM SPSS Statistics, Armonk, NY, USA) and the R

Table 1. Selected hematological and plasma biochemistry results from white-fronted Amazon parrots (*Amazona albifrons*) combined for both sexes. These values are based on data with normal distributions.

Parameter	N	Min	Max	Mean ± SD	CV
Erythrocytes, $\times 10^6$/L ($\times 10^{12}$/L)[a]	36	1.6 (1.6)[a]	1.6 (4.3)[a]	3 ± 0.5 (3 ± 0.5)[a]	0.19
MCV, fL	35	(109)[a]	(233)[a]	(154 ± 26.3)[a]	0.17
Total proteins, g/dL (g/L)[a]	37	2.1 (21)[a]	3.6 (36)[a]	2.9 ± 0.4 (28.8 ± 3.6)[a]	0.12
Albumin, g/dL (g/L)[a]	36	0.2 (2)[a]	1.3 (13)[a]	0.7 ± 0.2 (6.8 ± 2.5)[a]	0.37

Abbreviations: N indicates number; Min, minimum; Max, maximum; SD, standard deviation; CV, coefficient of variation; MCV, mean corpuscular volume.
[a] Values expressed in international units.

Program (v4.1.2; R Foundation for Statistical Computing, Vienna, Austria) to determine the reference intervals using the nonparametric bootstrap. $P < 0.05$ was used to determine statistical significance.

RESULTS

Thirty-seven healthy white-fronted Amazon parrots (21 males, 16 females), with an average weight of 199 g (SD±16.28, range 162–238 g), were sampled for this study. An average of 495 μL of blood was collected from each bird. The number of samples included in the hematological and biochemical results are listed in Tables 1–5.

The hematological and biochemical results for both sexes were combined as a single reference interval for each analyte based on their distribution (Table 1, normally distributed; Table 2, not normally distributed). A high CV was observed between individuals for both hematological and biochemical plasma parameters. Thrombocytes, cell morphology, and artifacts were not included in the statistical analysis due to the subjective nature of the data. No eosinophils were measured in these birds. Unfortunately, some biochemical values (2 GDH, 1 albumin) could not be measured due to insufficient sample volume. Four additional samples were excluded from the analysis due to clotting.

No statistically significant differences were found for any of the hematological or biochemical analytes by sex (all $P > 0.05$). Reference interval limits for parrots of both sexes yielded 90% confidence for the quantiles 2.5%, 50%, and 97.5%, obtained by nonparametric bootstrap with 1000 repetitions (Table 3). A CV below 10% was considered normal variability. Leukocytes, heterophils, lymphocytes, monocytes, basophils, uric acid, albumin, and GDH showed high variability. Erythrocytes, MCV, AST, and total protein showed lower variability in females and males (Table 4 and 5), as did the combined values for both sexes (Tables 1 and 2).

DISCUSSION

Hematological and selected biochemical reference intervals were determined in a captive colony of white-fronted Amazon parrots. Results from this study are consistent with findings in humans, birds, and dogs, and demonstrate biological

Table 2. Selected hematological and plasma biochemistry results from white-fronted Amazon parrots (*Amazona albifrons*) combined for both sexes. These values are based on data with non-normal distributions.

Parameter	N	Min	Max	Median	Mean ± SD	CV
PCV % (L/L)[a]	36	35 (0.35)[a]	56 (0.56)[a]	47.5 (0.47)[a]	46.8 ± 4.9 (0.5 ± 0.1)[a]	0.10
Total solids, g/dL (g/L)[a]	36	3 (30)[a]	4.4 (44)[a]	3.9 (39)[a]	3.8 ± 0.39 (38.2 ± 3.9)[a]	0.10
Leukocytes, cells/μL ($\times 10^9$/L)[a]	35	1100 (1.1)[a]	5700 (5.7)[a]	2200 (2.2)[a]	2511 ± 1183.2 (2.5 ± 1.2)[a]	0.47
Heterophils, cells/μL ($\times 10^9$/L)[a]	36	60 (0.1)[a]	3000 (3)[a]	700 (0.7)[a]	890 ± 756 (0.9 ± 0.7)[a]	0.84
Lymphocytes, cells/μL ($\times 10^9$/L)[a]	36	250 (0.2)[a]	2700 (2.7)[a]	1175 (1.2)[a]	1232.8 ± 531.2 (1.2 ± 0.5)[a]	0.43
Monocytes, cells/μL ($\times 10^9$/L)[a]	34	0 (0)[a]	400 (0.4)[a]	100 (0.1)[a]	145 ± 93 (0.1 ± 0.1)[a]	0.60
Basophils, cells/μL ($\times 10^9$/L)[a]	33	0 (0)[a]	400 (0.4)[a]	100 (0.1)[a]	140.9 ± 100.7 (0.1 ± 0.1)[a]	0.71
Uric acid, mg/dL (μmol/L)[a]	36	3.5 (206)[a]	12.7 (758)[a]	6.3 (375.5)[a]	6.9 ± 2.7 (408.7 ± 158.8)[a]	0.38
GDH, U/L	36	(1)[a]	(5)[a]	(2)[a]	(2.5 ± 0.8)[a]	0.33
AST, U/L	36	(99)[a]	(257)[a]	(151)[a]	(157.2 ± 38.6)[a]	0.24

Abbreviations: N indicates number; Min, minimum; Max, maximum; SD, standard deviation; CV, coefficient of variation; PCV, packed cell volume; GDH, glutamate dehydrogenase: AST, aspartate aminotransferase.
[a] Values expressed in international units.

Table 3. Reference intervals for selected hematological and plasma biochemistry parameters from white-fronted Amazon parrots (*Amazona albifrons*) combined for both sexes. The table represents the 90% confidence intervals of the 2.5%, 50%, and 97.5% obtained by 1000 bootstrap replications.

Parameter	N	2.5% (90% CI)	50% (90% CI)	97.5% (90% CI)
PCV%	36	36 (35–41)	47.5 (46–48)	55 (51–56)
[L/L][a]		[0.4 (0.3–0.4)][a]	[0.5 (0.5–0.5)][a]	[0.5 (0.5–0.6)][a]
Erythrocytes, $\times 10^6$/L	36	2 (1.6–2.5)	3 (3–3.3)	3.9 (3.5–4.3)
[$\times 10^{12}$/L][a]		[2 (1.6–2.5)][a]	[3 (3–3.3)][a]	[3.9 (3.5–4.3)][a]
MCV, fL	35	113.2 (109–119.4)	150 (144–155)	198.2 (185.9–233)
Total solids, g/dL	36	3 (3–3.4)	3.9 (3.7–4)	4.4 (4.2–4.4)
[g/L][a]		[30 (30–33.6)][a]	[39 (37–40)][a]	[44 (42–44)][a]
Leukocytes, cells/μL	35	1100 (1100–1300)	2200 (2000–2400)	5360 (4200–5700)
[$\times 10^9$/L][a]		[1.1 (1.1–1.3)][a]	[2.2 (2–2.4)][a]	[5.4 (4.2–5.7)][a]
Heterophils, cells /μL	36	180 (60–290)	700 (500–700)	2740 (2200–3000)
[$\times 10^9$/L][a]		[0.2 (0.1–0.3)][a]	[0.7 (0.5–0.7)][a]	[2.7 (2.2–3)][a]
Lymphocytes, cells /μL	36	560 (250–690)	1175 (1000–1300)	2440 (1780–2700)
[$\times 10^9$/L][a]		[0.6 (0.3–0.7)][a]	[1.2 (1–1.3)][a]	[2.4 (1.8–2.7)][a]
Monocytes, cells/μL	34	17 (0–65)	100 (100–200)	320 (300–400)
[$\times 10^9$/L][a]		[0 (0–0.1)][a]	[0.1 (0.1–0.2)][a]	[0.3 (0.3–0.4)][a]
Basophils, cells/μL	33	0 (0–41)	100 (100-–100)	320 (300–400)
[$\times 10^9$/L][a]		[0 (0–0.04)][a]	[0.1 (0.1–0.1)][a]	[0.3 (0.3–0.4)][a]
Plasma Biochemistry				
Parameter	N	2.5% (90% CI)	50% (90% CI)	97.5% (90% CI)
Uric acid, mg/dL	36	3.5 (3.5–3.9)	6.3 (5.6–7.3)	12.2 (10.6–12.7)
[μmol/L][a]		[208.6 (206–230.9)][a]	[375.5 (333.5–432)][a]	[726.5 (628.1–758)][a]
GDH, U/L	36	1 (1–2)	2 (2–3)	4.1 (3.1–5)
AST, U/L	36	103.4 (99–115)	151 (144.9–158)	251.7 (191.7–257)
Total proteins, g/dL	37	2.2 (2.1–2.4)	2.8 (2.8–3)	3.5 (3.3–3.6)
[g/L][a]		[21.9 (21–23.9)][a]	[28 (28–30)][a]	[35.1 (33.1–36)][a]
Albumin, g/dL	36	0.3 (0.2–0.4)	0.7 (0.6–0.8)	1.2 (1–1.3)
[g/L][a]		[2.9 (2–3.8)][a]	[7 (6–8)][a]	[12.1 (10–13)][a]

Abbreviations: N indicates number; CI, confidence interval, PCV, packed cell volume; MCV, mean corpuscular volume; GDH, glutamate dehydrogenase; AST, aspartate aminotransferase.

[a] Values expressed in international units.

variation between individuals of the same species.[21–24] In humans, hematological variation can reach 40% due to intraindividual circadian cycles, gender differences, and certain environmental variables (eg, smoking).[23,24] Biochemical analyte concentrations can also vary in birds and humans between age groups, sex, circadian cycles, and annual rhythms.[11,25–27]

In this study, the blood samples were collected only in the winter. Season has been shown to affect hematological and biochemical parameters in some bird species. For example, AST activity increases in the Northern Hemisphere winter, whereas calcium concentrations decrease in the same months for budgerigars (*Melopsittacus undulatus*) in the Southern Hemisphere.[26] This has been attributed to the budgerigar reproductive cycle.[26] Seasonal hematological variation has also been documented in some avian species in winter[28,29] and early spring[30] compared to summer. Snow geese (*Anser caerulescens*) and Canada geese (*Branta canadensis*) exhibit elevated PCVs, erythrocyte counts, and hemoglobin concentrations in winter and early spring.[30] American kestrels (*Falco sparverius*) exhibit maximum PCVs in winter.[29] In another study, brown thornbill (*Acanthiza pusilla*), superb fairywren (*Malurus cyaneus*), red-browed firetail (*Emblema temporalis*), and eastern yellow robin (*Eopsaltria australis*) showed increased erythrocyte concentrations and decreased MCV, but no significant PCV variability in winter compared to summer values.[28] These changes are attributed to a metabolic response to cold stress, variation in seasonal reproductive hormones, migration, molting, and photoperiod.[28–30] Seasonal variability in other psittacines species is well documented,[26] but was not examined in this study and should be considered for future research.

Table 4. Selected hematological and plasma biochemical values for male and female white-fronted Amazon parrots (*Amazona albifrons*). These values are based on data with normal distributions.

	Female				
Variable	N	Min	Max	Mean + SD	CV
PCV, % (L/L)[a]	15	35 (0.3)[a]	56 (0.6)[a]	46.8 ± 0.04 (0.5 ± 0.04)[a]	0.09
Erythrocytes, $\times 10^6/\mu L$ ($\times 10^{12}/L$)[a]	15	2.1 (2.1)[a]	4.3 (4.3)[a]	3 ± 0.5 (3 ± 0.5)[a]	0.17
MCV, fL	15	(114)[a]	(233)[a]	(159.2 ± 28.4)[a]	0.17
Lymphocytes, cells/μL ($\times 10^9/L$)[a]	15	600 (0.6)[a]	2400 (2.4)[a]	1285 ± 551 (1.3 ± 0.5)[a]	0.42
Uric acid, mg/dL (μmol/L)[a]	16	3.6 (214)[a]	12.7 (758)[a]	7.5 ± 3 (449 ± 180.6)[a]	0.4
Total proteins, g/dL (g/L)[a]	16	2.1 (21)[a]	3.6 (36)[a]	2.9 ± 0.4 (28.7 ± 4)[a]	0.1
Albumin, g/dL (g/L)[a]	15	0.3 (3)[a]	1 (10)[a]	0.7 ± 0.2 (6.7 ± 2.1)[a]	0.3

Abbreviations: N indicates number; Min, minimum; Max, maximum; SD, standard deviation; CV, coefficient of variation; CI, confidence interval; PCV, packed cell volume; P, observed statistical significance; MCV, mean corpuscular volume.
[a] Values expressed in international units.

Individual biological variation of analytes due to homeostatic adjustments is well documented[31,32] and may affect the interpretation of a patient's hematological and biochemical test results.[31] The reference value of change and the individuality index distinguish and define a relationship between the values of the individual against an entire population.[33] In veterinary medicine, these criteria recently have been used to evaluate or determine reference values and validate their quality. This method has been applied to dogs,[22] budgerigars,[21] and bald eagles (*Haliaeetus leucocephalus*),[34] where intra- and interindividual variation, critical differences, biological variation, and individuality were considered.

Wide variability in the results was found in this study of 27 birds. According to the criteria of American Society for Veterinary Clinical Pathology Quality Assurance and Laboratory Standards, a minimum sample size of 120 individuals is desirable. However, at least 40 individuals may be acceptable if robust statistical methods are used. At least 40 female and 40 male individuals are required to determine the effect of sex. Limited sample sizes may reduce the accuracy and precision of the results,[20] and was thus a limitation of this study. A cross-sectional study with a larger sample size, and correlations between age, sex, and season, may provide more accurate reference values for white-fronted Amazon parrots.

There were no significant differences in the hematological or plasma biochemical parameters found between male and female white-fronted Amazon parrots in this study. Several avian species have been reported to exhibit variability in their hematological or plasma biochemical results between the sexes.[10,12] Changes in complete blood counts and serum biochemistry analytes in the horned guan have been attributed to reproductive changes, including an increase in calcium concentrations in females due to egg production and a higher MCV in males due to increased testosterone production.[10] In Galapagos penguins, males were found to have higher PCVs while females had higher eosinophil counts.[12] For the differences between sexes noted for the Galapagos penguins,

Table 5. Selected hematological and plasma biochemical values for male and female white-fronted Amazon parrots (*Amazona albifrons*). These values are based on data with non-normal distributions.

	Females					
Variable	N	Min	Max	Median	Mean ± SE	CV
Total solids, g/dL (g/L)[a]	15	3 (30)[a]	4.4 (44)[a]	4 (40)[a]	3.9 ± 0.3 (39.1 ± 3.4)[a]	0.08
Leukocytes, cells/μL ($\times 10^9/L$)[a]	15	1100 (1.1)[a]	5700 (5.7)[a]	2200 (2.2)[a]	2640 ± 1309 (2.6 ± 1.3)[a]	0.49
Heterophils, cells/μL ($\times 10^9/L$)[a]	15	200 (0.2)[a]	2700 (2.7)[a]	700 (0.7)[a]	1002 ± 851 (1 ± 0.8)[a]	0.84
Monocytes, cells/μL ($\times 10^9/L$)[a]	15	70 (0.1)[a]	400 (0.4)[a]	100 (0.1)[a]	178 ± 103 (0.2 ± 0.1)[a]	0.73
Basophils, cells/μL ($\times 10^9/L$)[a]	13	0 (0)[a]	300 (0.3)[a]	100 (0.1)[a]	131 ± 86 (0.1 ± 0.1)[a]	0.65
GDH, U/L	16	1	4	3	2.5 ± 0.8	0.32
AST, U/L	16	99	243	143	144.1 ± 37.1	0.26

Abbreviations: *P*, observed statistical significance; N, number; Min, minimum, Max, maximum; SE, standard error; CV, coefficient of variation; GDH, glutamate dehydrogenase; AST, aspartate aminotransferase.
[a] Values expressed in international units.

Table 4. Extended.

Variable	Male					
	N	Min	Max	Mean + SD	CV	*P*
PCV, % (L/L)[a]	21	36 (0.4)[a]	55 (0.5)[a]	46.7 ± 0.05 (0.5 ± 0.05)[a]	0.11	0.93
Erythrocytes, $\times 10^6/\mu L$ ($\times 10^{12}/L$)[a]	21	1.6 (1.6)[a]	3.8 (3.8)[a]	3.1 ± 0.5 (3.1 ± 0.5)[a]	0.16	0.74
MCV, fL	20	(109)[a]	(192)[a]	(150.1 ± 24.7)[a]	0.16	0.31
Lymphocytes, cells/μL ($\times 10^9/L$)[a]	21	250 (0.2)[a]	2700 (2.7)[a]	1195 ± 527 (1.2 ± 0.5)[a]	0.44	0.62
Uric acid, mg/dL (μmol/L)[a]	20	3.5 (206)[a]	10.3 (616)[a]	6.3 ± 2.3 (376.5 ± 135.1)[a]	0.35	0.18
Total proteins, g/dL (g/L)[a]	21	2.2 (22)[a]	3.5 (35)[a]	2.9 ± 0.3 (28.8 ± 3.4)[a]	0.11	0.96
Albumin, g/dL (g/L)[a]	21	0.2 (2)[a]	1.3 (13)[a]	0.7 ± 0.3 (6.9 ± 2.8)[a]	0.41	0.89

no explanation or reason was provided by the authors.[12] Male burrowing parrots have higher heterophil and lymphocyte counts than females.[13] Female budgerigars have been found to have higher AST activities and lower calcium concentrations than males, which has been associated with increased male activity and a lack of egg production.[26] No significant differences in hematological or serum biochemistry values were noted between sexes of mute swans or red-lored Amazon parrots.[7,14] Variability in hematological and biochemical parameters between sexes may be both species specific and influenced by the reproductive status of the animals when the samples are collected.[7,10–13,26]

Some previously reported hematological and biochemical reference intervals in *Amazona* and other psittacine birds are inconsistent with the results obtained in this study.[3–9,35,36] The most dramatic difference was a lower total leukocyte count, including eosinophils and heterophils, compared with other avian species.[4–6,8,35,36] The birds included in this study were clinically healthy, with no hematological inflammatory indicators, such as toxic or immature heterophils.[19] Mallards (*Anas platyrhynchos*) show biochemical and hematological changes, such as leukopenia, in late spring during molting and prior to migration.[37] The birds in the present study were not molting. Eosinopenia is difficult to confirm in birds[19] because in many cases the lowest reported reference value is zero.[4,7–9,13,35,36] An eosinophil reference value of zero has also been reported for healthy Cuban Amazon parrot and Cuban parakeet (*Aratinga euops*) hatchlings and juveniles.[35] Similar to other white blood cells, eosinopenia may be attributed to stress or administration of glucocorticoids.[19] However, the birds in this study did not receive immunosuppressive agents before or during the study[19] and were carefully handled to avoid stress. Variations in the leukogram can also be the result of normal and individual physiological fluctuations. To our knowledge, no interspecific statistical differences have been found in other studies investigating *Amazona* species' hematological and biochemistry panels;[3,4] however, very few psittacine hematological and biochemical studies sample a large population of the same species. Future meta-analyses may identify more accurate hematological and biochemical values for psitta-

Table 5. Extended.

Variable	Males						
	N	Min	Max	Median	Mean ± SE	CV	*P*
Total solids, g/dL (g/L)[a]	21	3 (30)[a]	4.4 (44)[a]	3.8 (38)[a]	3.7 ±0.4 (37.5± 4.1)[a]	0.1	0.25
Leukocytes, cells/μL ($\times 10^9/L$)[a]	20	1100 (1.1)[a]	5300 (5.3)[a]	2000 (2.0)[a]	2415 ± 1104 (2.4 ± 1.1)[a]	0.45	0.52
Heterophils, cells/μL ($\times 10^9/L$)[a]	21	60 (0.1)[a]	3000 (3)[a]	700 (0.7)[a]	809 ± 690 (0.8 ± 0.7)[a]	0.85	0.7
Monocytes, cells/μL ($\times 10^9/L$)[a]	19	0 (0)[a]	300 (0.3)[a]	100 (0.1)[a]	118 ± 77 (0.1 ± 0.1)[a]	0.65	0.09
Basophils, cells/μL ($\times 10^9/L$)[a]	20	10 (0.01)[a]	400 (0.4)[a]	100 (0.1)[a]	147 ± 110 (0.1 ± 0.1)[a]	0.74	1
GDH, U/L	20	2	5	2	2.5 ± 0.9	0.35	0.6
AST, U/L	20	122	257	153.5	167.7 ± 37.3	0.22	0.053

cine species. Hematological parameter variability has also been observed in psittacine chicks younger than 3 months of age,[38] suggesting ontogenic changes in blood counts as in other species.[14,35]

Albumin concentrations measured in this study were similar to those published for various cockatoo species[39,40] and *Eclectus roratus*[41] when measured by automatic analyzers. However, when albumin was measured by electrophoresis, higher values were obtained than when measured by spectrophotometry.[39,40] The albumin values published in this study were determined using bromocresol green, which is considered an unacceptable technique in some avian and mammalian species. Electrophoresis is the preferred method for measuring albumin and has been reported as the reference standard,[42–45] but it was not available in this study.

In this study, hematological and plasma biochemical reference values were partially established for white-fronted Amazon parrots using a captive population. No statistically significant sex differences were found, and many analytes had large CV. Their precision should be treated with caution because this study included only a limited number of birds. Future studies should consider a cross-sectional design with a larger sample size that considers various physiological and environmental factors (eg, age, sex, season) to determine the exact variability of reference values.

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