HEMOGRAM AND CLINICAL BLOOD BIOCHEMISTRY OF MACAWS (*Ara* sp.) IN ECOLOGICAL FARMS MAINTAINED BY THE STATE OF BAHIA, BRAZIL

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ABSTRACT

This research aimed to establish reference values for hemogram and clinical biochemistry, aiming at evaluating the liver function of healthy macaws (*Ara sp*.), verifying the influence of species on the blood constituents, in order to contribute to the health monitoring of this species kept in ecological sites in the state of Bahia (Brazil). We used forty-five blood samples from three distinct species: green-winged macaw (*Ara chloroptera*), blue-and-yellow macaw (*Ara ararauna*) and scarlet macaws (*Ara macao*). A total of 3.5 mL of blood was collected from the ulnar vein of each bird: 1.5 mL was placed in tubes containing EDTA, to carry out the hemogram, and 2.0 mL of blood without anticoagulant was used to obtain serum, for the biochemical analysis. The averages obtained for the blood of the genus *Ara* were PCV: 2.74 ± 0.48 x10^6/µL; GV: 36.8 ± 5.56%; Hb: 15.4 ± 2.42 g/dL; thrombocytes:9,580 ± 2,850 x10^3/µL; leukocyte count: 5,340 ± 3,580/µL; lymphocytes: 1,408.8 ± 1,020.7/µL; heterophils: 3,252.0 ± 2,026.3/µL; monocytes: 169.1 ± 227.5/µL; basophils: 34.2 ± 72.3/µL; eosinophils: 186.9 ± 183.9/µL. The results of the serum biochemical parameters were activity of the enzymes AST 67.6 ± 12.8 IU/L; CK 77.9 ± 44.6 IU/L; LDH 240.1 ± 85.6 IU/L; cholesterol 160.3 ± 34.8 mg/dL; triglycerides 111.8 ± 58.6 mg/dL; glucose 228.5 ± 38.1 mg/dL and total proteins: 3.37 ± 0.8 g/dL. Significant differences ($p < 0.05$) influenced by species were detected for the values of number of PCV, GV, number of leukocytes and the concentration of cholesterol and glucose.

KEYWORDS: Biochemistry; Haemogram; *Psittacidae*.

HEMOGRAMA E BIOQUÍMICA CLÍNICA SANGUÍNEA DE ARARAS (*Ara sp.)* MANTIDAS EM SÍTIOS ECOLÓGICOS NO ESTADO DA BAHIA

RESUMO

Esta pesquisa visou a estabelecer os valores de referência do hemograma e de parâmetros de bioquímica clínica, com fins de avaliar a função hepática de araras (*Ara sp*.) sadias, verificando-se a influência da espécie sobre os constituintes sanguíneos, de forma a contribuir com o monitoramento da saúde desses psitacídeos mantidos em sítios ecológicos no Estado da Bahia. Foram utilizadas 45 amostras de sangue de três espécies distintas: *Ara ararauna* (arara canindé, n=29), *Ara macao* (arara piranga, n=9) e *Ara chloroptera* (arara vermelha, n=7). Coletaram-se de cada ave 3,5 mL de sangue da veia ulnar, sendo 1,5 mL colocados em tubos contendo EDTA, para a realização do hemograma, e 2,0 ml, sem anticoagulante para obtenção de soro, destinados às provas de bioquímica. As médias obtidas para o hemograma do gênero *Ara* foram hemácias totais 2,74 ± 0,48 x10^6/µL; VG 36,8 ± 5,56%; Hb: 15,4 ± 2,42 g/dL; trombócitos: 9.580 ± 2.850 x10^3/µL; contagem de leucócitos: 5.340 ±

3.580/µL; linfócitos: 1.408,8 ± 1.020,7/µL; heterófilos: 3.252,0 ± 2.026,3/µL; monócitos: 169,1 ± 227,5/µL; basófilos: 34,2 ± 72,3/µL; eosinófilos: 186,9 ± 183,9/µL. Os resultados dos parâmetros da bioquímica sérica foram: atividade das enzimas AST 67,6 ± 12,8 UI/L; CK 77,9 ± 44,6 UI/L; LDH 240,1 ± 85,6 UI/L; colesterol 160,3 ± 34,8 mg/dL; triglicérides 111,8 ± 58,6 mg/dL; glicose 228,5 ± 38,1mg/dL e proteínas totais: 3,37 ± 0,8 g/dL. Verificou-se a influência da espécie, representada pelas diferenças significativas (p<0,05) para os valores do número de hemácias, volume globular, do número de leucócitos e da concentração de colesterol e glicose.

PALAVRAS-CHAVE: bioquímica clínica; hemograma; *Psittacidae*.

INTRODUCTION

Psittacines are birds that belong to the family Psittacidae and are represented by macaws, parakeets and parrots. In Brazil, six species of true macaws are known: *Anodorhynchus hyacinthinus*, *Anodorhynchus leari*, *Anodorhynchus glaucus* (extinct), *Ara ararauna*, *Ara macao* and *Ara chloroptera*. There is also a large number of species kept in captivity, not only as pets, but also in zoos and preservation sites (STORM, 1996).

The successful establishment of psittacines rearing in captivity depends on management practices, as well as on the control and treatment of diseases, since these birds are susceptible to various diseases such as hepatopathies, parasitic infections, neoplasias and intoxication. Furthermore, they are important sources for the infection by *Chlamydophila psittaci* to humans (BENEZ, 2001).

The veterinary clinic has an unique role in the context of animal health and it is necessary to use indicators to assess the physiology of these individuals in order to allow the veterinarian to establish the clinical diagnosis of many different diseases that affect wildlife, make predictions, institute therapies and apply preventive measures.

The evaluation of hematological and biochemical parameters is important for the diagnosis of diseases, thus preventing the spread of disease between bird-man and even among the stock. Biochemical analyzes in blood serum or plasma of animals present results that are fundamental for the clinical diagnosis of liver diseases and these tests are assembled in batteries of tests referred to as liver function evaluators.

The following tests are recommended for birds: protein profile, enzymatic activity of aspartate aminotransferase (AST) and creatine kinase (CK). This assessment should be complemented with glucose and cholesterol determination (SANTOS, 1999). However, in order to properly use such tests, the physiological constants in different species and in their respective regions should be known (CORDEIRO, 2003).

In Brazil, reference values for the hemogram of psittacides are those established in other countries where the climate, region, nutrition and management are different from those found here, leading to errors in the interpretation of results. Thus, there is a need for studies on the values obtained in healthy animals and for the evaluation of the influence of variability factors (SANTOS, 1999).

Among the factors causing physiological variability in blood constituents of birds, we highlight those related to species, sex, age, reproductive management and nutrition, among others, being the hemogram parameters scarce in Brazilian literature (CARDOSO & TESSARI, 2003.)

Therefore, this study aimed to establish normality values of the constituents of hemogram and blood biochemistry, in order to assess the liver function in healthy macaws *(Ara sp.)*, and to investigate the influence of the species on these parameters, contributing to monitoring the health of these birds, kept in ecological sites in the State of Bahia, Brazil.

MATERIAL AND METHODS

We used 45 adult macaws with average weight of 350 g including three different species: green-winged macaw *(Ara chloroptera)* (n = 7), blue-and-yellow macaw *(Ara ararauna)* (n = 29) and scarlet macaw *(Ara macao)* (n = 9), of which, initially, 37 came from the Instituto Planeta Zôo, located in the city of Lauro de Freitas. Five months after the beginning of the collection, this Institute was transferred to Getulio Vargas zoobotanic park in the city of Salvador, and then, eight blue-and-yellow macaws, coming from the preservation breeding site, also located in the city of Salvador, were included in the sample of birds used for this research. All birds were adult, clinically healthy and identified with individual bird banding. Due to the transfer, the newly-arrived birds underwent a quarantine period at the zoo, with the purpose of acclimating the animals to their new environment, compatible with IBAMA standards.

Before the beginning of the material collection, the birds were submitted to clinical examinations, according to RUPLEY (1999), and parasitological tests were also carried out, and the identification of eggs / larvae was done according to GREINER & RITCHIE (1994), in order to evaluate the animals´ healthiness, including in this study clinically healthy and free of gastrointestinal parasites specimens.

All birds were maintained according to standards for breeding birds in captivity, receiving zoo-sanitary care and fed *ad libitum* with a diet consisting of tropical fruits, psittacines feed (AM16, Megazoo) and seeds, standardized in the place of origin of the macaws .

All ethical principles recommended for the use of animals in experiments were observed (CEUA-MEV da Universidade Federal da Bahia), and approved by the Ethics Committee of the institution under number 20/2011.

The birds were divided into three groups, according to the species, and two blood samples were taken from each macaw, by venipuncture of the ulnar vein on the ventral surface of the umer-radioulnar joint (CAMPBELL, 1994), with an interval of 15 to 20 days between the collections, using physical restraint of animals. All samples were taken at the same time, between seven o'clock and ten o'clock in the morning. At the first collection, 1.5 mL of blood was obtained, blood smears were immediately made and glucose measurement on portable glucometer (Accu-Chek Advantage, Roche®) was carried out. Next, the remaining blood was placed into tubes containing the trisodium ethylenediamine tetraacetic acid (EDTA k3) 10%, for the complete blood count and the determination of total plasma protein, which was performed within 12 hours of collection.

At the second collection, 2.0 mL of blood were collected and centrifuged to separate serum, the samples were stored at -20°C for seven days, when the biochemical analyses were performed.

Samples obtained from both collections were kept under refrigeration and transferred to the Laboratory of Parasites of Domestic Animals, at Escola de Medicina Veterinária da Universidade Federal da Bahia where they were processed.

For the blood test, 37 blood samples were used, and the count of the total number of red blood cells and leukocytes was performed in a modified Neubauer chamber, according to the Natt & Herrick technique (CAMPBELL, 1994; CARDOSO & TESSARI, 2003; LANZAROT et al., 2005). The result of the number of red blood cells was expressed as x106/μL and the number of leukocytes count was expressed in /μL (BENEZ, 2001). The determination of hemoglobin (Hb) was performed by the cyanmethemoglobin method, and globular volume (GV) by the microhematocrit (Ht) method. The results were expressed in g/dL and as percentage (%), respectively. The RBC indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as recommended by Wintrobe (MATOS & MATOS, 1981; CAMPBELL, 1994) and expressed, in fL, pg and g/dL, respectively.

Differential counts of leucocytes and thrombocytes were held in fresh blood smears stained with Roosenfeld stain and then the types of leukocytes and thrombocytes were identified by the morpho-staining characteristics. The results of thrombocytes and leukocytes are presented in cells /μL.

Biochemical parameters were determined by the use of Doles commercial "kits" and reading with spectrophotometer (HITACHI, 2000). The serum activity of the enzymes aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH) were determined by the colorimetric method and read at a wavelength of 505 nm, 660nm and 510nm, respectively. Triglycerides and cholesterol were obtained by the Trinder enzymatic method using the wave length of 510nm, and total serum protein (PPT) was obtained by using biuret method, at a wavelength of 550nm. All determinations were carried out following the kits manufecturer´s recommendation. The colorimetric method using the biuret technique is extremely accurate for determination of plasma protein in serum or plasma (CAMPBELL, 1994)

The means, standard deviations, medians and confidence intervals (with 95% significance) of each variable were determined for each species and for the genus. The Mann-Whitney test with significance ($p < 0.05$) was applied for comparison among species. All data was processed using SPSS software version 15.0.

RESULTADOS E DISCUSSÃO

The data of the constituents of the erythrocyte count, leukocyte count and serum chemistry obtained in this research for the species *Ara ararauna*, *Ara chloroptera* and *Ara macao* and for the genus *Ara* are found in Tables 1, 2 and 3 and in Figures 1, 2 and 3.

Table 1. Values (mean, standard deviation, median and confidence interval) of the constituents of the erythrocyte, thrombocyte and the total plasma protein count, of blue-and-yellow macaw *(Ara ararauna, n = 23)*, green-winged macaw *(Ara chloroptera, n = 7)* and scarlet macaw *(Ara macao, n = 7)* and *Ara* genus (n = 37), kept in ecological sites in the metropolitan region of Salvador, Bahia, 2009

		Statistical Data			
Parameter	Species	$\overline{X}$	$\overline{SD}$	MED	CI
RBC (x10^6/µL)	*Ara ararauna*	2.56 [a]	0.53	2.61	2.3-2.8
	Ara chloroptera	2.98 [b]	0.24	2.90	2.7-3.2
	Ara macao	2.96 [b]	0.34	3.08	2.7-3.2
Genus	*Ara sp.*	2.74	0.48	2.82	2.5-2.9
GV (%)	*Ara ararauna*	34.5 [a]	5.26	34.0	32.1-36.8
	Ara chloroptera	40.8 [b]	3.23	42.0	37.8-43.8
	Ara macao	39.1 [b]	5.27	38.0	35.0-43.2
Genus	*Ara sp.*	36.8	5.56	36.0	34.9-38.6
Hb (g/dL)	*Ara ararauna*	14.9 [a]	2.38	14.9	13.8-16.0
	Ara chloroptera	16.4 [a]	1.21	16.6	15.3-17.6
	Ara macao	15.7 [a]	3.08	15.49	13.3-18.0
Genus	*Ara sp.*	15.4	2.42	15.49	14.6-16.2
MCV (fL)	*Ara ararauna*	138.5 [a]	27.5	132.1	125.9-151.0
	Ara chloroptera	137.9 [a]	19.7	137.0	119.6-156.2
	Ara macao	132.9 [a]	20.8	119.4	116.9-149.0
Genus	*Ara sp.*	137.0	24.24	134.1	128.9-145.1
MCH (pg)	*Ara ararauna*	59.6 [a]	10.38	57.3	54.9-64.3
	Ara chloroptera	55.5 [a]	6.77	57.4	49.2-61.8
	Ara macao	53.9 [a]	14.07	51.7	43.1-64.7
Genus	*Ara sp.*	57.4	10.87	56.9	53.8-61.0
MCHM (g/dL)	*Ara ararauna*	43.6 [a]	5.35	43.6	41.1-46.0
	Ara chloroptera	40.4 [a]	3.02	38.6	37.6-43.2
	Ara macao	40.1 [a]	6.20	41.4	35.3-44.9
Genus	*Ara sp.*	42.1	5.36	42.1	40.3-43.9
Thrombocytes (/µL)	*Ara ararauna*	9,800 [a]	3,070	9,100	8,400-11,190
	Ara chloroptera	8,950 [a]	2,440	8,000	6,700-11,200
	Ara macao	9,560 [a]	2,830	10,100	7,400-11,740
Genus	*Ara sp.*	9,580	2,850	9,100	8,630-10,530

RBC=Red blood cells; GV= Globular Volume; Hb=hemoblobin concentration; MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Hemoglobin; MCHC= Mean Corpuscular Hemoglobin Concentration / X= Mean; SD= Standard Deviation; MED= Median; CI= Confidence interval of 95%. In the column for each parameter, different letters correspond to values with significant difference ($p<0.05$).

Table 2. Values (mean, standard deviation, median and confidence interval) of the constituents of the leukogram of blue-and-yellow macaw *(Ara ararauna, n = 23)*, green-winged macaw *(Ara chloroptera, n = 7)* and scarlet macaw *(Ara macao, n = 7)* and *Ara* genus (n = 37), kept in ecological sites in the metropolitan region of Salvador, Bahia, 2009

		Statistical Data			
Parameter	Species	$\overline{X}$	$\overline{SD}$	MED	CI
Leu (/µL)	*Ara ararauna*	4,640 [a]	3,650	3,300	2,900-6,300
	Ara chloroptera	7,570 [b]	3,500	7,000	4,200-10,800
	Ara macao	5,230 [ab]	3,010	4,500	2,900–7,500
	Ara sp.	5,340	3,580	4,000	4,100–6,500
Lym (/µL)	*Ara ararauna*	1, 188.7 [a]	913.30	775.0	772.9-1,604.5
	Ara chloroptera	1,863.0 [a]	1,190.1	1,575.0	762.2-2,963.7
	Ara macao	1,569.3 [a]	1,097.2	1,300.0	725.9-2,412.7
	Ara sp.	1,408.8	1,020.7	1,215.0	1,068.5-1,749.2
Het (/µL)	*Ara ararauna*	3,116.8 [a]	2,396.5	2,046.0	2,025.9-4,207.6
	Ara chloroptera	3,650.0 [a]	2,309.9	2,720.0	1,513.6-5,786.3
	Ara macao	3,258.1 [a]	1,832.6	2,880.0	1,849.4-4,666.7
	Ara sp.	3,252.0	2,026.3	2,565.0	2,516.4-3,987.6
Mon (/µL)	*Ara ararauna*	180.1 [a]	272.8	75.0	55.9-304.3
	Ara chloroptera	151.5 [a]	161.7	115.0	1.96-301.1
	Ara macao	157.2 [a]	162.8	108.0	32.0-282.3
	Ara sp.	169.1	227.5	93.0	93.2-245.0
Bas (/µL)	*Ara ararauna*	6.4 [a]	17.6	0.0	0.0-14.4
	Ara chloroptera	62.0 [a]	101.5	24.0	0-155.8
	Ara macao	77.7 [a]	101.0	0.0	0.09-155.4
	Ara sp.	34.2	72.3	0.0	10.1 – 58.4
Eos (/µL)	*Ara ararauna*	155.4 [a]	167.3	100.0	79.2-231.6
	Ara chloroptera	302.0 [a]	266.2	160.0	55.7-548.2
	Ara macao	170.8 [a]	120.7	180.0	78.0-263.7
	Ara sp.	186.9	183.9	120.0	125.6 – 248.2

Leu=Leukocytes; Lym=Lymphocytes; Het=Heterophils; Mon=Monocytes; Bas=Basophils; Eos=Eosinophils / X= Mean; SD= Standard Deviation; MED= Median; CI= Confidence interval of 95%. In the column for each parameter, different letters are statistically different ($p<0.05$).

Table 3. Values (mean, standard deviation, median and confidence interval) of the constituents of the blood biochemistry of blue-and-yellow macaw *(Ara ararauna, n = 29)*, green-winged macaw *(Ara chloroptera, n = 7)* and scarlet macaw *(Ara macao, n = 9)* and *Ara* genus (n = 45), kept in ecological sites in the metropolitan region of Salvador, Bahia, 2009

		Statistical Data			
Parameter	Species	$\overline{X}$	$\overline{SD}$	MED	CI
AST (UI/L)	*Ara ararauna*	67.9 [a]	15.2	72.0	61.4-74.5
	Ara chloroptera	67.5 [a]	3.00	66.0	62.7–72.3
	Ara macao	64.1 [a]	14.9	66.0	50.3-77.93
	Ara sp.	67.6	12.8	72.0	62.4-72.7
CK (UI/L)	*Ara ararauna*	86.4 [a]	48.4	67.7	65.4-107.3
	Ara chloroptera	64.4 [a]	17.0	60.7	37.3-91.5
	Ara macao	73.6 [a]	43.3	61.0	33.6-113.7
	Ara sp.	77.9	44.6	63.4	59.8-95.9
LDH (UI/L)	*Ara ararauna*	233.5 [a]	74.4	221.4	193.8-273.2
	Ara chloroptera	346.7 [a]	117.9	412.0	53.7-639.7
	Ara macao	204.3 [a]	67.2	230.8	133.8-274.8
	Ara sp.	240.1	85.6	229.7	204.7-275.4
Cholesterol (mg/dL)	*Ara ararauna*	198.7 [b]	64.7	195.3	170.7-226.7
	Ara chloroptera	144.9 [a]	18.2	143.3	115.9-173.9
	Ara macao	146.6 [a]	26.3	141.8	122.3-171.0
	Ara sp.	160.3	34.8	154.7	146.3-174.4
Triglycerides (mg/dL)	*Ara ararauna*	99.2 [a]	57.9	89.0	70.4-128.0
	Ara chloroptera	114.7 [a]	24.9	102.8	83.8-145.7
	Ara macao	136.6 [a]	58.2	117.1	88.7-178.4
	Ara sp.	111.8	58.6	102.4	88.1-135.5
Glucose (mg/dL)	*Ara ararauna*	229.5 [ab]	39.6	235.0	213.8-245.2
	Ara chloroptera	216.0 [a]	12.9	218.5	195.3-236.7
	Ara macao	243.7 [b]	17.7	236.0	227.3-260.0
	Ara sp.	228.5	38.1	229.5	213.1-243.9
TPP (g/dL)	*Ara ararauna*	3.43 a	0.67	3.4	3.14-3.72
	Ara chloroptera	3.50 a	0.38	3.6	2.89-4.10
	Ara macao	3.32 a	1.05	3.1	2.35-4.29
	Ara sp.	3.37	0.8	3.4	3.0-3.7

AST= Aspartate Aminotransferase; CK=Creatine-kinase; LDH=Lactate Dehydrogenase; TPP= Total Plasma Protein / X= Mean; SD= Standard Deviation; MED= Median; CI= Confidence interval of 95%. In the column for each parameter, different letters are statistically different ($p<0.05$).

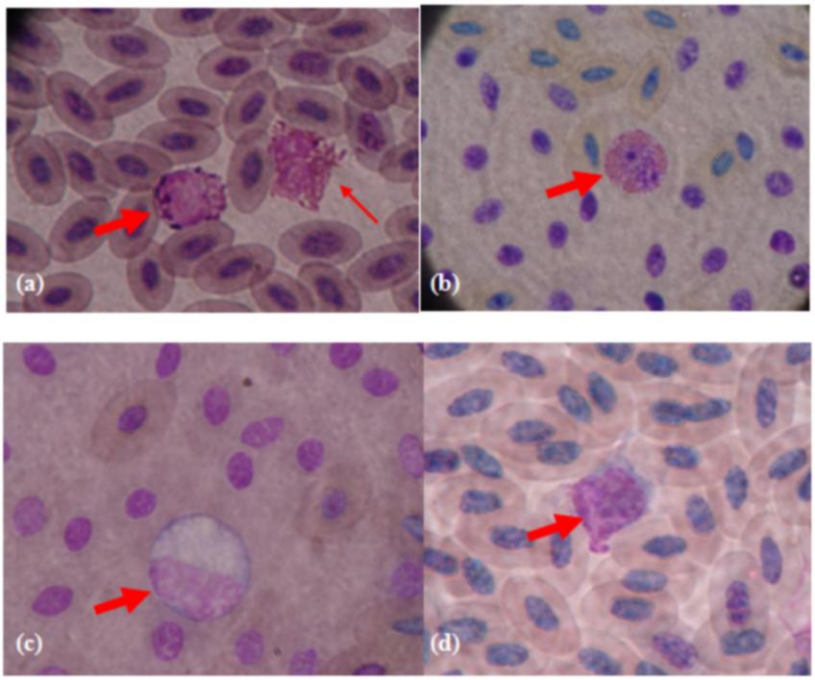

Figure 1. Blood cells of *Ara sp.* kept in ecological sites in the metropolitan region of Salvador, Bahia. (a) basophil (wide arrow) and heterophil (narrow arrow); (b) eosinophil; (c) lymphocyte and (d) monocyte.

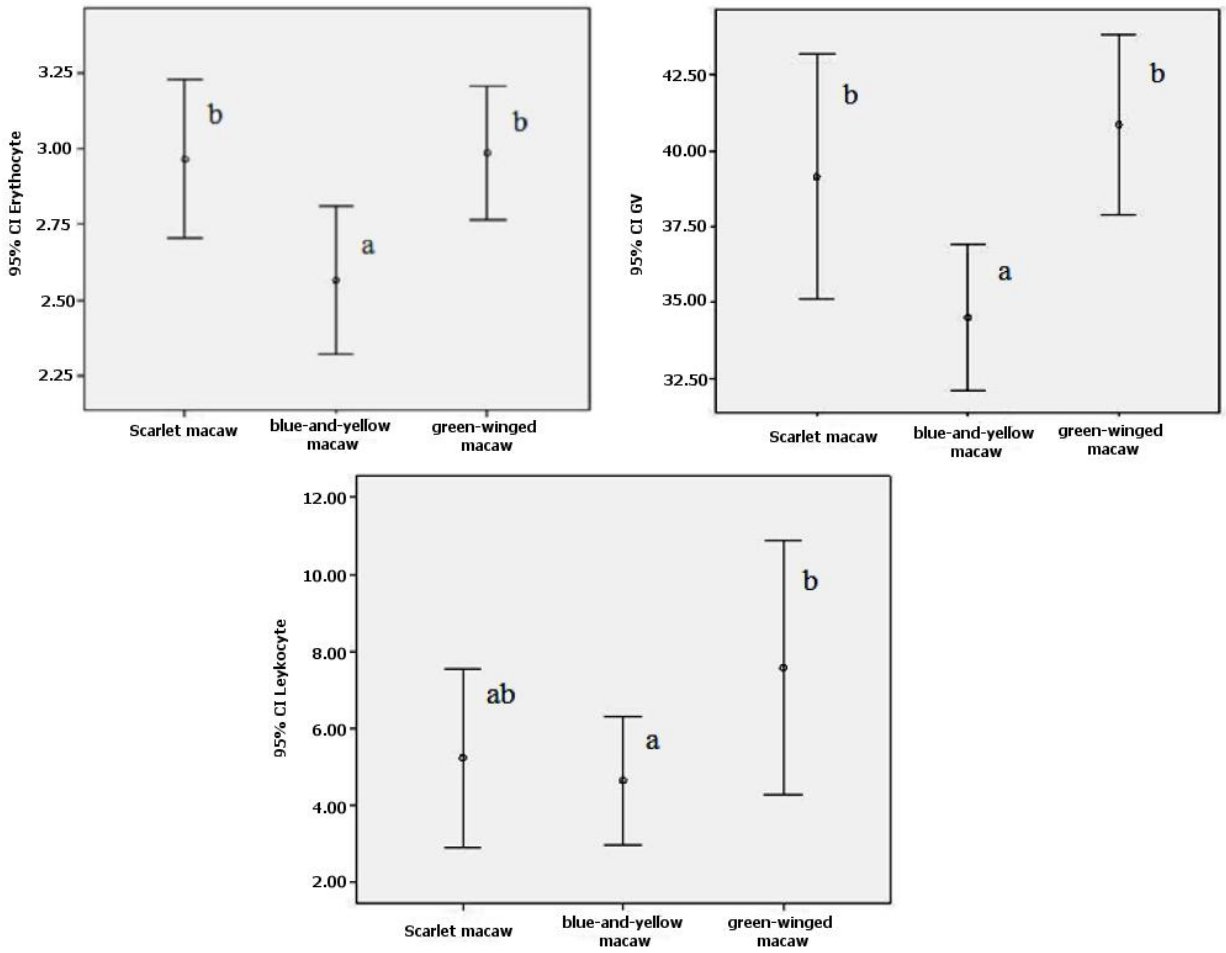

Figure 2. Means (± standard deviation) of the variables RBC, GV and Leu of *Ara sp*. kept in ecological sites in the metropolitan region of Salvador, Bahia. Different letters are statistically significant ($p<0.05$, Mann-Whitney test).

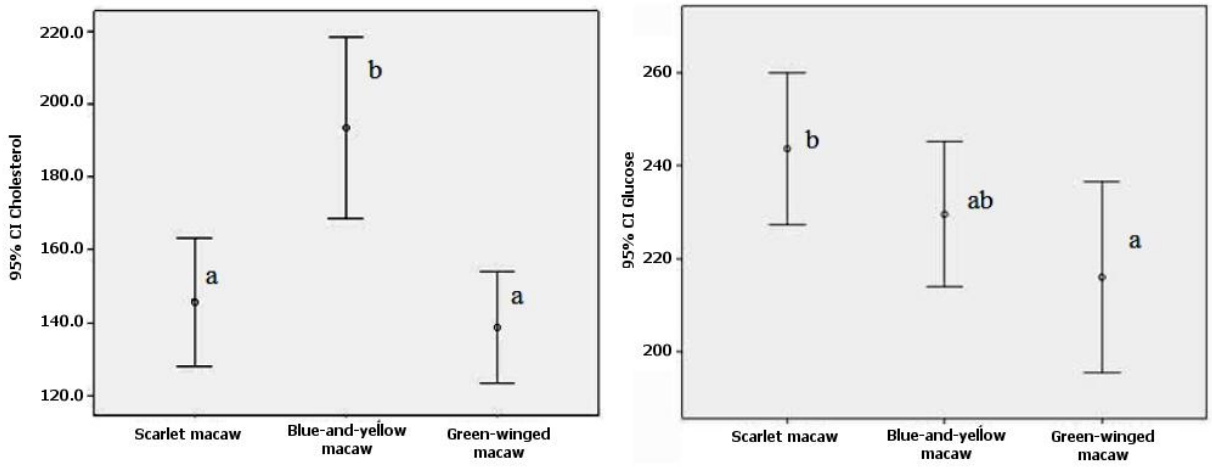

Figure 3. Means (± standard deviation) of glucose and cholesterol of *Ara sp*. kept in ecological sites in the metropolitan region of Salvador, Bahia. Different letters are statistically significant ($p<0.05$, Mann-Whitney test).

In the comparative analysis of erythrocyte count results there were no significant differences among the macaw species, for the total number of red blood cells and globular volume. Blue-and-yellow macaws showed significantly lower values for the number of red blood cells (2.56 ± 0.53 x 106/µL) and globular volume (34.5 ± 5.26%) when compared with scarlet macaws (2.96 ± 0.34 x 106/µL and 39.1 ± 5.27%) and green-winged macaws (2.98 ± 0.24 x 106/µL and 40.80 ± 3.23%). In the leukogram assessment, the total number of leukocytes from the blue-and-yellow macaw group obtained value of 4,640 ± 3,650/µL, which is significantly lower than the values found in the green-winged macaw group (7,530 ± 3,500/µL). These results characterize the species influence on the variables of the hemogram of the genus *Ara*.

The values of the hemogram variables in this study are within the intervals described for other wild bird species (DRIVER, 1981; GARCIA-MONTIJANO, 2002) and psittacines (GARCIA-DEL-CAMPO et al., 1991; FOLDENAUER et al., 2007). In the particular case of the genus *Ara*, the results of the number of red blood cells and hemoglobin concentration (2.74 ± 0.48 x106/µL and 15.4 ± 3.80 g/dL) were similar to the values determined by SANTOS (1999), which were 2.21 ± 0.45 x106/µL and 15.89 ± 1.55 g/dL; however, other studies have established greater values (POLO et al., 1998; BONELLO et al. 2002).

The globular volume value obtained in each macaw group in this research and the overall mean for the genus *Ara* (36.8 ± 5.6%) were lower than those reported by other authors, who also studied this genus (POLO et al., 1998; SANTOS, 1999; BONELLO et al., 2002; ALLGAYER et al., 2005). However, the analysis of the confidence interval found in this study (35.0 to 43.2%) showed that this is in accordance with the intervals reported by other studies from 31.5 to 54.0% and from 42.0 to 53.0%, respectively (POLO et al., 1998; BONELLO et al., 2002). Statistically significant differences ($p <0.05$) observed among the macaw groups showed the influence of species on the hemogram parameters, which was also verified by POLO et al (1998).

Regarding the results of the RBC indices, the value obtained for the MCV is within the one reported by BONELLO et al. (2002) and POLO et al. (1998), who found similar intervals (102.4 to 199.1 fL). The value of MCHC (42.1 ± 5.36 g/dL) was also within the established by POLO et al. (1998), which ranged from 31.3 to 61.9 g/dL, but was higher than the one found by ALLGAYER et al. (2005), which was equal to 26.5 g/dL. As one would expect, the differences in the values of the number of red blood cells and globular volume used in these calculations reflect the values of RBC indices.

The values found in the thrombocyte count showed no statistically significant differences among the macaw groups, and the value obtained for the genus *Ara* (9,500 ± 2,850 /µL) cannot be compared with other surveys conducted in psittacines, because this variable has not been assessed in these birds; however, HOWLETT et al. (1998) studied the hematological profile of *Ardeotis kori* and found similar values, which was equal to

7030 ± 1790 / uL.

In the leukogram evaluation, total counts showed significant differences among the macaw groups, which was also detected by POLO et al. (1998); nevertheless, while those authors obtained the lowest value in the green-winged macaw group, this group had the highest average in this present study (7,570 ± 3,500 / uL). The values found here for the genus *Ara* is close to that reported by other authors who also established the means of the components of the leukocyte count in the same genus of psittacines (SANTOS, 1999; ALLGAYER et al., 2005), 4,500 ± 3,080/µL and 5,700 ± 1,523.5 /µL, respectively.

In the assessing of leucocytes differential count, heterophils, followed by lymphocytes, were found in greater number, which is in accordance with other studies (HAWKEY et al., 1984; CAMPBELL, 1994; POLO et al., 1998; SANTOS, 1999; ALLGAYER et al., 2005). However, the absolute value of the lymphocytes in this study was lower (1,408.8 ± 1,020.7/uL) than that verified by ALLGAYER et al. (2005), who determined values of 2,734.3 ± 769.4 µL, and lower absolute values for heterophils (3,045.9 ± 1,003.5/µL), monocytes (63 ± 12.7/µL) and eosinophils (72 /µL) than we did in this study. Furthermore, those authors emphasized they did not report the existence of basophils in the blood smears of psittacines.

This variation found mainly in heterophils count may be due to the restraint method used, since in the present study, the birds were physically restrained, which can cause a high level of stress, thus increasing the number of heterophils.

Regarding the morphostaining characteristics of the blood cells, red blood cells are in accordance with the description by CAMPBELL (1994) and variations in color, size and shape of erythrocytes were observed in smears from healthy macows as described by other authors (MATOS & MATOS, 1981; RUPLEY, 1999); however, most of these cells presented oval and polychromatic shape, in both young and mature, as described by GOULART (2006).

As for leukocytes, heterophils were easily identified, showing bright rod-shaped granules, different from eosinophils, whose cytoplasm contained spherical granules of uniform size, with dense chromatin staining intensity (HAWKEY et al., 1984a). Thrombocytes (oval, and nucleated and smaller than red blood cells) were generally found near those cells, agglutinated and, due to its color and small size, they were easily identified, not being confused with small lymphocytes, as described by FUDGE (2000).

The use of Rosenfeld method for staining blood smears in this study must be emphasized because it has not been reported by any of the authors included in this comparative analysis, although the method has shown better color and more evident cells differentiation than the fast panoptic method (Instat-Prov), mentioned by BONELLO et al. (2002), making easier the identification of blood cells of psittacides.

Considering the comparative analysis of the results and the differences highlighted for some components of the blood count of psittacines, climate, nutrient management and the type of breeding seem to be some of the determinants of this variability, since the surveys were conducted in other regions (POLO et al., 1998; SANTOS, 1999; BONELLO et al., 2002; ALLGAYER et al., 2005; VALLE et al., 2008), but the techniques employed, often using different diluent solutions, also lead to different results.

In the assessment of serum biochemical parameters, significant differences were found between species for the cholesterol concentration, and the blue-and-yellow macaw group presented higher mean value (198.7 ± 64.7 mg/dL) than scarlet macaw group (146.6 ± 26.3 mg/dL) and green-winged macaw group (144.9 ± 18.2 mg/dL). In addition, significant differences were found for glucose concentration between the green-winged macaw group, which had the lowest value (216.0 ± 12.9 mg/dL), and the scarlet macaw group, with the highest value (243.7 ± 17.7 mg/dL). Among the variables studied, these results showed that the species may be a factor of variability on cholesterol and glucose among the psittacines of the genus Ara.

Regarding the biochemical parameters, the mean values of AST (67.6 ± 12.8 IU / L) showed no statistical significant differences among the macaw groups and were superior to those mentioned by the authors who have studied psittacines of *Amazona aestiva* species (DEEM et al., 2005) and the genus *Ara* in different countries (LUMEIJ & OVERDUIN & 1990; CRAY et al., 2008) and in Brazil (VALLE et al., 2008); however, these parameters agree with the studies by POLO et al. (1998), for blue-and-yellow macaws (56.2 ± 19.1 UI/L) and scarlet macaws (68.1 ± 24.1 UI/L), but lower value was obtained in the green-winged macaw group (49.0 ± 11.2 UI/L) by these authors.

The values determined for CK in the macaw groups included in this study did not differ significantly and were close to those determined by POLO et al. (1998) for blue-and-yellow macaws (86.9 ± 45.5 UI / L) and green-winged macaws (64.0 ± 41.9 UI/). However, these results differed from those obtained in the scarlet macaw group

studied by POLO et al. (2008), who obtained higher mean value (131.0 ± 109 UI/L), as well as from other researches that established value of 117.0 ± 119 UI/L for the genus *Ara* (LUMEIJ & OVERDUIN, 1990) and from confidence intervals, which were higher in other studies (CRAY et al., 2008; VALLE et al., 2008), respectively, 100-300 UI / L and 112.5-200.3 IU / L.

Factors related to the restrain methods and lean body mass of the birds in such researches may have influenced the results, increasing the CK activity (LUMEIJ, 1997). Considering the much higher values obtained for other psittacines (BAILEY et al., 1998; DEEM et al., 2005; FOLDENAUER et al., 2007) and wild birds (VILLEGAS et al. 2004), the metabolism of CK activity is also influenced by species and flight activity carried on by them (CRAY et al., 2008).

In the LDH analysis, the means were not significant among the macaw groups in this study and the confidence interval (95%) is within the one found by CRAY et al. (2008), which was 120-300 IU/L. Despite these results being close to the established by POLO et al. (1998) for scarlet macaws (226 ± 91.6 IU/L), there is a great variation between the maximum and minimum values (61.7 to 610 IU/L) in the genus *Ara*, the object of their study, an observation also made by LUMEIJ & OVERDUIN (1990), whose findings ranged between 193-483 IU/L. According to KRAMER (1989), there is a large concentration of this enzyme in red blood cells, therefore, the results from different authors may occur due to the possibility of hemolysis, even in small quantities, in the serum of birds.

Generally in birds, many variability factors affect the enzymes for the evaluation of liver function, especially age, nutritional management and collection method (HOCHLEITHNER, 1994, BAILEY et al., 1998).

The result of cholesterol mean concentration (160.3 ± 34.8 mg / dL) determined for the genus *Ara* showed a value within the confidence interval (150.1 mg/dL and 215.7 mg/dL) obtained for blue-and-yellow macaws (VALLE et al., 2008). Statistically significant differences ($p < 0.05$) observed among the macaw groups in this research, where blue-and-yellow macaws presented the highest value (198.7 ± 64.7 mg / dL) compared with green-winged macaws (144, 9 ± 18.2 mg / dL) and scarlet macaws (146.6 ± 26.3 mg / dL), are also close to the interval mentioned above.

It is worth noting that during the individual data analysis, differences were seen when comparing the value obtained in macaws kept in a zoo in Salvador (173.88 ± 46.41 mg/dL) with those of the ecological breeding site (242.5 ± 77.7 mg/dL), values consistent with the results reported by VALLE et al. (2008). This was attributed to the fasting state of birds kept in zoos, which did not occur with the birds from the breeding site, where the collection was done after feeding, besides the differences in the diets offered to the macaws. Those kept in the zoo were fed a mixture of fruit, a small amount of commercial diet for psittacines and seeds (sunflower and corn), being the latter ingredients supplied two or three times a week, whereas the birds kept in ecological breeding site were fed with commercial feed (suitable for macaws) and on alternate days they were given green coconut.

In literature, we found no information about the nutritional needs of these birds; however, SAAD et al. (2007) verified that parrots *(A. aestiva)* use the more palatable components of the diet, rejecting the others and therefore do not absorb all the nutrients they need, although they are present in the diet. The difference of the value obtained for another psittacines for the cholesterol rate (354.0 ± 42.8 mg / dL) allowed us to affirm that this blood constituent differs among species and genus (FOLDENAUER et al., 2007).

The value of triglycerides concentration obtained in this study (111.8 ± 58.6 mg / dL) for *Ara sp.* showed no statistically significant differences among the groups, agreeing with the values verified by POLO et al. (1998), who found 106.8 ± 62.3 mg / dL, but this result was only determined for blue-and-yellow macaws. The macaws of the groups had similar weights and in psittacine species from Argentina *(Cyanolyseus patagonus)*, the influence of body condition on triglycerides concentrations was observed (MASELLO & QUILLFELDT, 2004). By analyzing the value obtained for the green-winged macaws group included in this study (136.6 ± 58.2 mg / dL), we observed that the result reported by POLO et al. (1998) was lower. These differences may suggest the species as a factor of variability.

The amount and type of inadequate lipids in the diet of birds have been documented in situations where high triglycerides blood concentrations can impair circulation and cause sudden death syndrome (LEESON, 1994; GONZALEZ, 2001). Moreover, the possibility of the presence of aflatoxins by the use of some types of seeds for feeding birds in captivity can predispose to liver diseases (ORSI et al., 2005), thus it was essential to establish this parameter for monitoring psittacines in captivity.

The glucose mean found for the macaws studied (228.5 ± 38.1 mg/dL) was lower than those established in other studies (LUMEIJ &

OVERDUIN, 1990; POLO et al., 1998; SANTOS, 1999), which were, respectively, 271.8 ± 37.8 mg / dL, 270 ± 30.6 mg / dL and 296.4 mg / dL. This difference can be justified by the fasting state of the birds surveyed, information which was not provided by the authors cited, besides the difference in methodology.

The portable glucometers have been used in veterinary medicine as a means to monitor blood glucose of animal in a variety of medical conditions (STEIN & GRECO, 2002) and have shown good correlation and accuracy compared with the standard laboratory tests (ALTO et al., 2002), although there are no reports of its use in birds. A significant difference ($p < 0.05$) among macaw species included in this experiment, in which green-winged and blue-and-yellow macaws had lower mean values (216.0 ± 12.9 mg/dL and 229.5 ± 39.6 mg/dL) when compared to scarlet macaws (243.7 ± 17.7 mg/dL). POLO et al. (1998) also found differences among species, but the highest values were obtained in groups of blue-and-yello and green-winged macaws (271.8 ± 37 mg/dL and 270 ± 30.6 mg/dL) and lower for the group of scarlet macaws (248.4 ± 16.2 mg/dL). This result was supported by the statements of CORDEIRO (2003), who verified that the psittacines diversity, associated with different ecological species nuclei present influences related to climate change and energy metabolism in birds with individual character.

The birds used in this study are not docile, since they live in large enclosures at the zoo and are not handled frequently; moreover, although they are accustomed to human presence, they do not tolerate closeness. Thus, the stress of the birds may have caused the differences between the compared data. Besides this fact, some of the researchers previously mentioned used the method of chemical restraint, which may have led to divergent data from those found in this work, where physical restraint method was used.

The values obtained for plasma proteins (3.37 ± 0.8 g / dL) are in agreement with the ones reported by VALLE et al. (2008), which determined a confidence interval (95%) from 3.5 to 3.8 g / dL and did not find difference among macaw groups, however, BONELLO et al. (2002) found higher value (4.9 g / dL). Possibly this is due to a diet richer in protein causing differences in protein metabolism of psittacines (LUMEIJ & OVERDUIN, 1990).

CONCLUSION

The species has an influence on the hemogram parameters of birds from the genus *Ara*, as well as on concentrations of cholesterol and glucose. In addition, the nutritional management can have an effect on cholesterol concentration.

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