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RESEARCH ARTICLE

Leukocyte profile of the helmeted manakin, *Antilophia galeata* (Passeriformes: Pipridae) in a Cerrado forest fragment

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ABSTRACT. Changes in the amounts and proportions of leukocytes, known as leucocyte profiles, have been documented for several bird species and have been used to measure stress levels in these animals. The present work ascertained the biological and ecological attributes that influence the leukocyte profile of *Antilophia galeata* (Lichtenstein, 1823), the helmeted manakin. This species has been deemed useful in ecological studies because it responds to environmental changes. Blood samples drawn from 89 individuals of *A. galeata* captured in a Cerrado forest fragment were subjected to analysis under optical microscopy to identify and quantify leukocytes and micronuclei. The number of lymphocytes was greater for males, non-reproductive individuals and individuals infected with ticks. None of the leukocyte components differed in relation to age, molting or body condition index. The amount of micronuclei was correlated with values for total leukocytes, H/L ratio, heterophils, basophils and monocytes. The results suggest that reproduction may be an immunosuppressive factor for the species, producing sexual differences in lymphocyte availability. In addition, biomarkers of genotoxic damage (micronuclei) were related to the amount of leukocytes, indicating that individuals may be sensitive to environmental disturbances. Leukocyte profiles can be considered a useful tool for addressing ecological questions that are relevant to the conservation of species in degraded environments.

KEY WORDS. Avian hematology, Brazilian birds, micronuclei, stress, white blood cells.

INTRODUCTION

Various biological and physiological parameters can be used to assess stress in birds, including hematological exams such as the evaluation of leukocyte profiles (Davis et al. 2008). Leukocyte profiles are determined by differential leukocyte counts and represent a major component of the vertebrate immune system, comprising various cell types that provide protection against pathogens and stressors (Campbell 2015, Maceda-Veiga et al. 2015).

Avian leukocytes can be classified into granulocytes and agranulocytes or mononuclear cells. The former include basophils, eosinophils and heterophils, while the latter include lymphocytes and monocytes (Campbell 2004, Fernandes et al. 2013). Lymphocytes undergo maturation in lymphoid organs, such as the thymus or bursa of Frabricius, and are responsible for acquired or adaptive immunity with the production of antibodies and specific responses to pathogens (Campbell 2015). Heterophils, eosinophils, basophils and monocytes act primarily on innate immunity, which is the first line of immune defense (Davison et al. 2008). Bird heterophils are equivalent to the mammalian neutrophils and are the major phagocytic cells involved in the inflammatory response, attacking bacteria through chemotaxis, opsonization, phagocytosis, and lysis (Cândido 2008, Capitelli and Crosta 2013). The functions of eosinophils and basinophils in birds are uncertain, but both may play a role in hypersensitivity reactions, while eosinophils may also act against parasitic infections (Kiesecker 2002, Mitchell and Johns 2008). Monocytes phagocytize and degrade microorganisms, abnormal cells and cellular debris (Campbell 2015).

Heterophils and lymphocytes together make up approximately 80% of the leukocytes of birds and their amounts and proportions can change according to variation in corticosteroid concentrations (Davis et al. 2008, Wojczulanis-Jakubas et al. 2015). When present at high levels, these hormones promote

greater recruitment of heterophils in relation to lymphocytes, thereby increasing the H/L ratio, which characterizes the leukocyte stress profile (Gross and Siegel 1983, Davis et al. 2008). This adjustment in the proportions of heterophils and lymphocytes occurs during stressful periods, when individuals tend to suppress one component of the immune system while enhancing another (Apanius 1998), as demonstrated by experiments with laboratory animals using exogenous treatment with synthetic glucocorticoids (Dhabhar et al. 1995).

Evidence has shown that the leukocyte profiles of birds differ between males and females, since sex hormones act as important modulators of the immune response: testosterone is immunosuppressive and estrogen is immune-enhancing (Campo and Davila 2002, Charles-Smith et al. 2014, Foo et al. 2016, Roved et al. 2017). Since young birds have yet to undergo sexual maturation, their leukocyte profiles may differ from those of adults. In addition, there are reports of higher H/L ratios during reproduction; that is, an indication of greater stress during the reproductive period, since, for both sexes, reproduction involves high energetic demands (Wojczulanis-Jakubas et al. 2015, Frigerio et al. 2017) such as during incubation and parental care, egg production for females and defense of territories for males (Saino et al. 2002, Greenman et al. 2005).

Birds undergo other stressors that may influence their leukocyte profile, such as parasitism, food shortage and molting, among others. Lüdtke et al. (2013) found that the H/L ratio is significantly higher in individuals of the Eurasian blackcap, *Sylvia atricapilla* (Linnaeus, 1758), infected with hemoparasites. Palacios et al. (2017) observed that molting individuals of the chinstrap penguin, *Pygoscelis antarctica* (J.R. Forster, 1781), have a greater proportion of leukocytes. Machado-Filho et al. (2010) found that individuals of the plain-crested elaenia, *Elaenia cristata* Pelzeln, 1868, had higher H/L ratios during the dry season in a savannah environment, which the authors attributed to stress due to scarcity of food. Other studies have recorded changes in the leukocyte profiles of birds in relation to environmental disturbances, such as pollution. Eeva et al. (2005) observed that individuals of the European pied flycatcher, *Ficedula hypoleuca* (Pallas, 1764), sampled in an area contaminated with copper, had higher H/L ratios. Bauerová et al. (2017) revealed a positive correlation between H/L ratio and the amount of lead, cadmium, copper and chromium present in the plumage of the Eurasian great tit, *Parus major* Linnaeus, 1758.

Another alteration evidenced in hematological examinations is the presence of erythrocyte micronuclei, which has been associated with anthropic influences (Baesse et al. 2015, 2019, Martínez-Haro et al. 2017). Micronuclei are small, round structures located in the cytoplasm. They have chromatic characteristics similar to those of the principal nucleus (Gattás et al. 1992). Their shape is due to factors or events that interfere with the structure or function of the mitotic apparatus, which produces failures in the incorporation of chromosomes or chromosomal fragments in the principal nucleus (Fenech et al. 2006, Thomas et al. 2009). Micronuclei reflect chromosomal damage and, therefore, can act as biomarkers of genotoxicity (Hitoshi et al. 2003, Baesse et al. 2015, 2019). Analysis of micronuclei can be used as an efficient method to assess mutagenicity of, and sensitivity to, environmental contaminants (Wolf et al. 2002, Baesse et al. 2019). Micronuclei and the H/L ratio can be viewed as relatively independent indicators of stress, and any association between the two would lend support to their use as physiological stress indicators. Furthermore, assessing two relatively independent stress indicators provides a far more comprehensive view of an animal's physiological stress profile than examining just one single indicator.

Few studies have documented the leukocyte components in Brazilian bird species, and the influence of stressful situations on them (Machado-Filho et al. 2010, Lobato et al. 2011). Brazil harbors one of the richest avifauna in the world with approximately 2,000 described species (Piacentini et al. 2015). The present study examined a population of the helmeted manakin, *Antilophia galeata* (Lichtenstein, 1823), a piprid passeriforme endemic to the Cerrado, and which inhabits the understory of riparian forests. The species can be found in preserved areas such as conservation units, and in altered sites, including urban environments such as city parks (Franchin and Marçal-Júnior 2004, Valadão et al. 2006, Silva and Melo 2011). The species is monogamous and mostly frugivorous, sometimes incorporating invertebrates in its diet (Silva and Melo 2011, Dantas 2013). Adult males have black plumage with red feathers on top of the head. Adult females and juveniles of both sexes have a discrete greenish plumage throughout the body (Marini 1992, Sick 1997).

The helmeted manakin has been considered useful in ecology studies because it is territorial, dependent on the forest environment and is sensitive to the effects of disturbances, being a species that can show responses to environment changes (Silva and Melo 2011, Gonçalves 2012, Dantas 2013, Teles 2013, Baesse et al. 2015, 2019, Paniago 2016). The present study proposed to examine the leukocyte profile of a population of *A. galeata* and to associate it with biological and ecological attributes of the species. The hypotheses tested are that the leukocyte stress profile should predominate in: (i) males, because they are territorial and aggressive and this behavior is associated with increased levels of the immunosuppressive hormone testosterone; (ii) juveniles, possibly due to greater sensitivity to stress; (iii) individuals of both sexes in the reproductive period, because of the high energy demands; (iv) individuals of both sexes during molting, since the renewal of feathers requires a lot of energy; (v) individuals infested with ticks, since the exploitive action of these parasites can cause stress in birds. It is also expected that individuals in poor body condition and with a greater quantity of micronuclei would display a leukocyte stress profile.

MATERIAL AND METHODS

The study was carried out in a Cerrado forest fragment at the Fazenda Experimental do Glória (18°57'03"S; 48°12'22"W),

in the municipality of Uberlândia, Minas Gerais, Brazil. The fragment encompasses 30 ha composed of seasonal semideciduous forest with gallery forest at is lower limit and abrupt transitions at artificial borders with pastures at the upper and lateral limits (Lopes 2010). The forest is adjacent to the highway BR 050, which is heavily trafficked by motor vehicles (Baesse et al. 2019). The climate of the region is Aw type, according to the climatic classification of Köppen, with a dry season (April-September) and a rainy season (October-March). Annual rainfall is around 1,500 mm, while the mean temperature is 22 °C (Rosa et al. 1991).

Seven field campaigns of five days each were undertaken from June 2013 to December 2015. Birds were captured using 20 to 25 mist nets (12 m long x 3 m high) exposed on trails between 6:30 am and 5:00 pm. The nets were checked at intervals of approximately 30 minutes. When birds were found they were removed and placed in clothe bags for subsequent screening. Individuals were weighed in the bags using a hand dynamometer (Pesola®), with the weight of the bag then being subtracted. The tarsal length of each bird was measured using a digital caliper (Lotus®). Individuals were identified and banded with metal bands provided by Center for Research and Conservation of Wild Birds (CEMAVE/ICMBio – Authorization: 3730, Registry: 359076).

The reproductive stage of the captured birds was determined by the presence of an incubation patch; when the abdominal region lacks feathers and has thin, wrinkled skin with a darker coloration due to increased vascularization (Cemave 1994). The feathers covering the abdominal region were blown to allow visualization of the incubation patch and determine its presence. In addition, feathers from other regions of the body (head, neck, back) were blown to detect the presence of ticks and molting (appearance of new feathers).

When possible, the age group (young or adult) of each individual was determined, since young birds usually have a yellowish labial commissure and the absence of, or minimal, cranial ossification. To determine sex, blood samples were colleted from all individuals of green color (05 µL of blood from the tarsal vein) with the aid of sterile disposable needles (8 × 0.3 mm) (SISBIO/ICMBio – Authorization: 44901). The collected blood samples were stored in specific kits provided by the sexing laboratory. The samples were sent to a private laboratory for molecular sexing (©Unigen Tecnologia do DNA – from São Paulo, SP, Brazil).

A drop of blood (05 µL) was collected from the tarsal vein and placed on a clean, sterile microscope slide (SISBIO/ICMBio – Authorization: 44901). With the aid of a second slide inclined at 45°, the blood was drawn across the first slide. After drying, the slides were fixed with absolute methanol while still in the field. In the laboratory the slides were stained with a solution of Giemsa (5%) and phosphate buffer (pH 5.8) for 30 minutes. The slides were then washed in distilled water, dried at room temperature and identified with the individual's band number.

Slides were analyzed under an optical microscope with a 100x objective using immersion oil, with 200 fields per individual (100 per slide). The components of the leukocyte profile were identified according to the descriptions of Clark et al. (2009) and Campbell (2015) provided in their respective atlases of avian hematology. In order to obtain total leukocyte count (TLC), the counts for each leukocyte type were summed, whereas the percentage of each type was obtained by dividing the count for each type by TLC. The H/L ratio was calculated by dividing the percentage of heterophils by the percentage of lymphocytes. All these calculations were done according to Machado-Filho et al. (2010).

Micronuclei analysis involved counting the number of micronuclei in each of the 5,000 erythrocytes on each slide for a total of 10,000 erythrocytes per individual (Baesse et al. 2015). Each erythrocyte can contain one or more micronuclei, which were identified according to the criteria proposed by Wolf and Luepke (1997).

The capacity of individuals to store resources and survive adverse situations can be evaluated by analyzing the body condition (Schulte-Hostedde et al. 2005). Body condition can be estimated by the Relative Mass Index (RMI), which was calculated by simple linear regression between the log-transformed values of right tarsus length and individual biomass. The residual values of the regression were used as RMI, with negative values representing poorer body conditions than positive values (Schulte-Hostedde et al. 2005).

Parametric tests (Student's t test and Pearson correlation) were performed to determine whether the components of the leukocyte profile differed among the analyzed variables (sex, age, reproductive period, molting, ectoparasites, body condition and micronuclei). To meet the assumptions of the tests, percentage data (heterophils, lymphocytes, eosinophils, basophils, monocytes and H/L ratio) were transformed (arcsine of the square root), while the continuous variables (TLC and micronuclei) were log-transformed. The analyses were conducted using Systat 10.2 software with a level of significance of $p < 0.05$.

RESULTS

The following numbers of individuals were captured at each year of the research: 2013, 15 individuals; 2014, 14 individuals (eight in February and six in July); 2015, 60 individuals (25 in June, 18 in August, 12 in October and five in December). In total, 89 individuals were captured, of which 40 (44.9%) were females, 43 (48.3%) were males. It was not possible to determine the sex of six (6.74%) individuals. The sample included 58 (65.1%) adults, 14 (15.7%) juveniles and 17 (19.1%) individuals for which the development phase could not be determined. The birds for which sex and development could not be estimated were disregarded from analyses involving sexes and age groups. A total of 26 (29.2%) individuals possessed an incubation patch while 63 (70.7%) did not. Indications of molting were present in 23 (25.8%) individuals and absent in 66 (74.1%). Ticks were found infesting 17 (19.1%) individuals, while 72 (80.8%) were

tick free. Micronuclei were present in 55 (61.7%) individuals and absent in 34 (38.2%).

The number of lymphocytes was greater for males (t = -2.001, df = 81, 0.049, Table 1, Fig. 1); for individuals without incubation patch (t = 2.565, df = 86, p = 0.012, Table 1, Fig. 2); and for those infested with ticks (t = -3.064, df = 86, p = 0.003, Table 1, Fig. 3). There were no differences for any of the leukocyte components with regards to age group (Table 2), molting (Table 2), and RMI (Table 3). There was a significant correlation between the number of micronuclei and TLC (r = 0.343, df = 43, p = 0.024), H/L (r = -0.273, df = 53, p = 0.044), heterophils (r = -0.335, df = 53, p = 0.012), basophils (r = 0.280, df = 53, p = 0.039) and monocytes (r = 0.277, df = 53, p = 0.041) (Table 3).

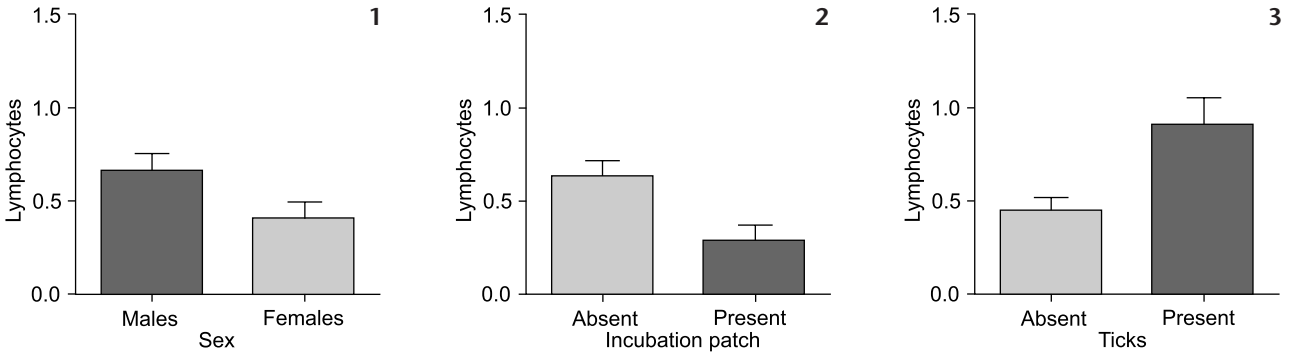
DISCUSSION

Although the leukocyte stress profile (H/L ratio) was not correlated to any of the analyzed variables, except for micronuclei, other components of the leukocyte profile varied according to the biological and ecological attributes of the individuals. The amount of lymphocytes was significantly greater in males, contrasting with what was expected: males were expected to have decreased amounts of leucocytes, consistent with studies that have shown that testosterone is an immunosuppressive hormone (Foo et al. 2016, Roved et al. 2017). Males of *A. galeata* are territorial and aggressive. In order to obtain a mate, they have to exhibit themselves for the females in large arenas where they fight each other in exhibitory flights (Marçal 2017). Studies have shown that territoriality is correlated with increased testosterone concentrations (Edler et al. 2011). Females, in contrast, had the lowest values of lymphocytes, suggesting that the activities that they perform, including incubation and caring for the nestlings, are more stressful (Marçal 2017). Other studies have also shown reduced amounts of lymphocytes in female birds (Hanssen et al. 2003, 2005), which was attributed to an immunosuppression associated with egg incubation. In the present study, individuals in charge of an incubation patch also had reduced lymphocytes. Although this structure occurs in both sexes of *A. galeata*, the present study found that 72% (n=25) of the individuals in charge of an incubation patch were females.

Differences in the number of lymphocytes were also associated with the presence of ectoparasites, with individuals with ticks having higher values. The number of lymphocytes was expected to be lower in infested individuals since, according to the reviews of Kotál et al. (2015) and Wikel (2017), several studies have found that proteins present in the saliva of ticks inhibit or reduce the proliferation of host lymphocytes. Of the infested individuals, 70.5% (n = 17) were males, which had a greater number of lymphocytes than females. This may be related to the absence of an immunosuppressive factor, such as incubation. Collectively, these numbers suggest that the variations in the number lymphocytes in these birds is more affected by reproduction than by the action of ticks.

Montolio et al. (2017) suggested that age is an important biological factor that can influence hematological variables, especially in early life when growth and development occur. They also reported greater amounts of leukocytes in juvenile owls, and suggested that this may be due to the fact that young individuals have greater sensitivity of to stress (Dickens et al. 2010). Nonetheless, there are also reports in the literature of adults having higher leukocyte values than juveniles. Campo and Davila (2002) found that the leukocyte levels of *Gallus gallus* (Linnaeus, 1758) increased continuously from the onset of sexual maturity. Schmidt et al. (2017) found greater values for heterophils, eosinophils and monocytes in adult pheasants, *Phasianus colchicus* Linnaeus, 1758, which were attributed to stress from reproductive events. No differences in leukocyte components were found between the age groups in the present study, suggesting that stress related to the allocation of resources for the growth of young can be equated to stress from reproduction in adults.

Molting is considered an energy demanding process, since it producing new feathers requires energy (Hemborg and Lundberg 1998). Individuals undergoing molting were expected to exhibit a leukocyte stress profile. Contrasting with this expectation, no effect of molting on the leukocyte profile was found. There have been other studies supporting our findings that



Figures 1–3. Number of lymphocytes for *Antilophia galeata* in relation to sex (1), presence/absence of incubation patch (2) and presence/absence of ticks (3). Boxes represent mean values while bars represent the standard error.

Table 1. Mean ± standard deviation for the components of the leukocyte profile of *Antilophia galeata* in relation to sex, reproductive period, and ticks. Statistically significant differences are shown in bold (t = Student's t test, df = degrees of freedom, p = significance probability < 0.05).

Hematologic parameters	Sex					Incubation patch					Ticks				
	Females (n = 40)	Males (n = 43)	Statistics			Present (n = 25)	Absent (n = 61)	Statistics			Present (n=17)	Absent (n=72)	Statistics		
			t	df	p			t	df	p			t	df	p
TLC	7.80 ± 9.88	8.74 ± 12.2	0.387	62	0.700	12.9 ± 11.9	6.06 ± 8.20	-0.229	66	0.766	6.23 ± 8.07	8.25 ± 10.1	0.122	66	0.904
H/L ratio	0.49 ± 1.50	0.71 ± 1.68	-1.929	81	0.057	1.55 ± 2.36	1.14 ± 4.66	-0.227	86	0.821	2.66 ± 8.44	0.88 ± 1.92	-1.012	86	0.314
Heterophils (%)	32.3 ± 37.6	24.7 ± 31.5	0.750	81	0.456	43.5 ± 28.8	26.9 ± 35.6	-1.482	86	0.142	22.8 ± 33.5	32.5 ± 34.4	1.323	86	0.188
Lymphocytes (%)	25.8 ± 36.0	37.8 ± 39.4	**-2.001**	**81**	**0.049**	32.3 ± 31.8	34.1± 39.9	**2.565**	**86**	**0.012**	27.6 ± 38.3	35.0 ± 37.8	**-3.064**	**86**	**0.003**
Eosinophils (%)	0.49 ± 1.50	0.77 ± 2.20	0.979	81	0.094	5.75 ± 10.8	0.80 ± 2.47	-0.992	86	0.324	0.49 ± 2.02	2.25 ± 7.03	*	*	*
Basophils (%)	0.90 ± 3.56	0.99 ± 2.60	-0.216	81	0.829	1.74 ± 5.65	0.72 ± 2.25	-0.884	86	0.379	1.07 ± 3.05	0.96 ± 3.13	*	*	*
Monocytes (%)	4.56 ± 13.8	2.67 ± 5.94	0.643	81	0.522	6.07 ± 16.6	2.08 ± 5.34	-0.057	86	0.178	2.04 ± 4.15	3.35 ± 10.7	*	*	*

Table 2. Mean ± standard deviation for the components of the leukocyte profile of *Antilophia galeata* in relation to age groups and molting (t = Student's t test, df = degrees of freedom, p = significance probability < 0.05, * = insufficient data for the test).

Hematologic parameters	Age group					Molting				
	Adult (n = 58)	Young (n = 14)	Statistics			Present (n = 23)	Absent (n = 66)	Statistics		
			t	df	p			t	df	p
TLC	14.4 ± 23.78	5.33 ± 6.17	0.543	55	0.589	7.60 ± 8.14	7.29 ± 10.3	-1.450	66	0.155
H/L ratio	1.54 ± 4.93	0.85 ± 1.52	0.452	70	0.652	0.49 ± 1.16	1.47 ± 4.64	0.427	86	0.671
Heterophils (%)	37.2 ± 65.1	29.5 ± 34.5	1.655	70	0.102	28.2 ± 30.8	31.5 ± 35.6	0.965	86	0.337
Lymphocytes (%)	30.8 ± 36.0	39.2 ± 40.5	-1.236	70	0.221	34.7 ± 38.1	33.2 ± 37.9	-1.335	86	0.185
Eosinophils (%)	2.66 ± 7.61	0.42 ± 1.52	1.099	70	0.276	2.35 ± 5.26	2.10 ± 6.82	-0.050	86	0.960
Basophils (%)	1.11 ± 3.45	–	*	*	*	2.10 ± 4.79	0.59 ± 2.16	-1.286	86	0.202
Monocytes (%)	4.05 ± 11.7	0.8 ± 2.91	*	*	*	7.92 ± 17.9	1.49 ± 3.75	-0.507	86	0.614

Table 3. Pearson correlations (r) between components of the leukocyte profile of *Antilophia galeta* and the Relative Mass Index (RMI) and the amount of micronuclei. Statistically significant correlations are shown in bold (df = degrees of freedom; p = probability of significance < 0.05).

Hematologic parameters	Correlation with RMI			Correlation with micronuclei		
	r	df	p	r	df	p
TLC	0.324	66	0.569	**0.343**	**43**	**0.024**
H/L ratio	0.047	86	0.665	**-0.273**	**53**	**0.044**
Heterophils (%)	-0.062	86	0.568	**-0.335**	**53**	**0.012**
Lymphocytes (%)	0.119	86	0.270	0.164	53	0.232
Eosinophils (%)	0.069	86	0.522	0.248	53	0.068
Basophils (%)	-0.078	86	0.471	**0.280**	**53**	**0.039**
Monocytes (%)	-0.074	86	0.495	**0.277**	**53**	**0.041**

there is no relationship between molting and leukocyte profile (Machado-Filho et al. 2010, Kulaszewicz et al. 2015, Włodarczyk et al. 2017). According to Barta et al. (2008), individuals in poor nutritional status may postpone or discontinue molting. This means that individuals entering the molting period may be in relatively good condition and under low physiological stress, and that even though molting may be stressful, the negative effect it causes may be counterbalanced by the favorable status of the birds entering the process (Włodarczyk et al. 2017).

Studies have shown that nutritional status or body condition may influence the leukocyte profile of birds, and that there is a negative relationships between the H/L ratio and body condition indexes; in other words, the higher the stress, the worse the body condition, and vice versa (Plischke et al. 2010, Gladbach et al. 2010, Krams et al. 2012, Jakubas et al. 2015, Włodarczyk et al. 2017, Zhao et al. 2017). However, no significant relationships were found between leukocyte components and body condition in the present study. It may be that the absence of such a relationship is indicative of insufficient stress to alter body condition, as previous studies also suggested (Ewenson et al. 2001, Greenman et al. 2005, Owen and Moore 2006, Machado-Filho et al. 2010).

Significant positive correlations were found between the quantity of micronuclei and TLC, percentage of basophils and percentage of monocytes. The formation of micronuclei occurs through spontaneous chromosomal changes or environmental disturbances, with findings of increased micronuclei levels due to anthropization (Pereira et al. 2013, Baesse et al. 2015, Martinez-Haro et al. 2017). Environmental changes also exert an influence on the leukocyte profile, as there are records of associations between heavy metal pollution and increased H/L ratio for various species of birds (Eeva et al. 2005, Bauerová et al. 2017). The relationships found in the present study between micronuclei and leukocytes may be attributed to a common

stressor, such as air pollution, since birds easily absorb gasses or particles present in the air, when accumulating a large volume of air in their body for flight (Brown et al. 1997). Furthermore, in the region of the present study, Baesse et al. (2019) found that birds living in forest areas near to the city presented higher micronuclei frequency as a function of the greater exposure to emission of gases originating from the heavy vehicular traffic.

Although TLC, basophil and monocyte percentages correlated positively with micronuclei in the present study, heterophil percentage and H/L ratio were negatively correlated with micronuclei quantity. Heterophils are the primary cells that proliferate during acute inflammatory responses (Harmon 1998), while erythrocyte turnover can take 25 to 45 days in birds (Jones 2015). Thus, it is probable that when erythrocytes containing micronuclei appear, heterophils have already passed through their highest peaks and, consequently, also the highest H/L ratios, suggesting that although the stressor may be the same, different responses to the stress may manifest at different times. However, the relationships between micronuclei and leukocytes in birds are not yet well described and understood, and more studies are needed to clarify them.

The present study was the first to examine the leukocyte profile of *A. galeata*, and to determine the aspects of the species' biology and ecology that influence the leukocytes produced by these birds. Certain components of the leukocyte profile were shown to vary in relation to certain characteristics and life periods of *A. galeata*. The results suggest that reproduction may be an immunosuppressive factor for the species, thereby producing sexual differences in lymphocyte availability for individuals. Furthermore, biomarkers of genotoxic damage (miocronuclei) were correlated with the H/L ratio, indicating that individuals may be sensitive to environmental disturbances. In this way, it can be concluded that leukocyte profiles are a useful tool for answering ecological questions that can be applied to the conservation of species in degraded environments.

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