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Research Article

Immune status of the Eurasian Collared dove *Streptopelia decaocto* in northeastern Algeria

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Abstract

Since its initial sighting in Algeria in 1994, the Eurasian Collared dove (*Streptopelia decaocto*) has exhibited invasive behavior, expanding its range gradually and consistently to the country's extreme south. An inventory of its health status has emerged, trying to respond more accurately to the question: does the immune status play a role in this invasion? Our investigation into the health of both nestlings and adults included identification and quantification of blood cells in nestlings before fledging, focusing on the evolution of total red blood cells (RBC) and white blood cells (WBC); Analysis of the bursal index and microscopic examination of the bursa. The study involved collecting blood smears from nestlings at different ages (5 days, 10 days, 15 days), and euthanizing individuals from each age group (5 days, 10 days, 15 days, 18-20 days, and adults), to extract the bursa for histological examination. This process aimed to ascertain the immune status of the species in Annaba (Northeastern Algeria). Our findings indicate a decline in immune competence with age, starting from an "Excellent" status in early life, deteriorating to "Poor" in later stages, and finally reaching "Bad" in adulthood. Although our results do not definitively confirm a compromised health status in the Eurasian Collared dove population studied, they suggest the need for further research to substantiate these preliminary findings.

Keywords: Eurasian Collared dove, *Streptopelia decaocto*, Annaba, health status, bursa of Fabricius.

Introduction

The remarkable spread of the Eurasian Collared dove from its native habitat in Asia Minor stands as a significant event in 20th-century ornithology. The phases of this expansion were meticulously documented in Europe, attributed to the keen observations and numerous publications by researchers (Eraud et al., 2011). Similarly, its southeastward spread was notable, establishing it as a common species in regions like Jordan (Andrews, 1995), and the northern Arabian Peninsula (Bergier et al., 1999). The dove's journey into North Africa began with Morocco in 1986, primarily through migration from the Iberian Peninsula (Franchimont, 1987). By 1995, it was sighted in Tunisia, likely originating from Sardinia, while Algeria recorded its first encounter with the species in 1994 (Benyacoub, 1998). The rapid proliferation of the Eurasian Collared dove post a period of latency is thought to be influenced by factors such as urbanization and climatic shifts, which potentially extend the breeding season (Crooks & Soule, 1999). Its dispersal pattern, predominantly northwestward, involved 'jump dispersal' where small, isolated populations ahead of the main distribution front expanded and merged (Fujisaki et al., 2010). The underlying biological mechanisms facilitating the swift expansion of the Eurasian Collared dove remain elusive. Hypotheses include genetic adaptability, successful integration into human-dominated landscapes, and a robust reproductive capacity (Gibbons et al., 1993). Notably, the species exhibited similar expansion dynamics in North America, Europe, and North Africa, unimpeded by significant dispersal barriers (Romagosa & Labisky, 2000; Belabed et al., 2013a; Belabed et al. 2013b; Bendjoudi et al., 2015). Parasitism and the ability to resist biological challenges have been considered critical factors in this expansion. Notably, a direct correlation exists between the extent of parasite infestation and dispersal distances. Successful colonization is contingent upon the species' capability to adapt to urban settings and establish stable, enduring populations, which may further evolve to thrive in such environments (Møller, 2008). In Algeria, the Eurasian Collared dove's high level of commensalism with humans, frequent breeding, versatile nesting habits, and sustainable establishment in urban areas, particularly in Annaba, present an intriguing case for examining the dynamics of its colonization process (Benyacoub, 1998; Belabed et al., 2013a; Belabed et al., 2012).

Dispersal can influence interactions with other species, such as predators and parasites, and these interactions may significantly impact the evolution of dispersal mechanisms (Boulinier et al., 2001). In 2004, researchers investigated the hypothesis that the evolution of long dispersal distances is associated with the significant impact of parasites on their hosts, thereby influencing the evolution of robust immune responses (Møller et al., 2004). These long dispersal distances might evolve in contexts where parasites severely affect reproductive success (Møller & Erritzøe, 2001). At the interspecific level, a positive correlation between dispersal distance and immune response was predicted in 2004, attributed to the direct relationship between parasite-induced mortality and the host's immune defense levels (Martin et al., 2001; Møller et al., 2004). This correlation provides evidence for the link between natal dispersal distance, breeding habitat specialization, and immune function components in birds, suggesting that parasites have played a

crucial direct or indirect role in shaping the dispersal distances in common European bird species, such as the Eurasian Collared dove (Møller et al., 2004).

Among the host resistance mechanisms, the immune system stands out as an exceptionally effective control method (Schoenle et al., 2018). To mitigate parasite impact, host species deploy various defense strategies, including immune system activation and enhanced healthcare practices. The avian immune system primarily comprises the thymus, bursa of Fabricius, spleen, mucosa-associated lymphoid tissues, bone marrow, and blood (Oláh & Vervelde, 2008). The bursa of Fabricius, uniquely found in birds, is a hollow, oval, chestnut-shaped sac located dorsally to the cloaca. It serves as the site for B-cell lymphopoiesis, lymphocyte maturation, differentiation, and the development of the antibody repertoire (Ifrah et al., 2017). The immune system in vertebrates integrates two closely related processes: innate and acquired immunity (Male et al., 2012). Investigating hematological indices in domestic and wild birds can offer crucial insights into the immune response and provide an objective assessment of health status (Lashev et al., 2009). These physiological parameters can reveal much about the animals' performance in their environment and the cumulative adverse effects of various factors on individuals. The heterophils to lymphocytes ratio (H/L ratio) in blood is commonly used to measure the stress level an animal experiences (Müller et al., 2011). Heterophils and lymphocytes, the primary circulating immune cells in birds, are particularly sensitive to natural stressors or stress hormone administration. Consequently, relatively high heterophil counts compared to lymphocytes are reliable indicators of elevated glucocorticoid levels (Davis et al., 2008). Furthermore, high H/L ratios have been linked to increased mortality rates in field studies (Krams et al., 2012). Given the vast range of animal species receiving veterinary attention, data about wild and exotic species are particularly significant. In certain instances, such findings may serve as indicators of disrupted ecological balance (Lashev et al., 2009). Notably, literature reveals a scarcity of published data on the hematological parameters of healthy Eurasian Collared doves (*Streptopelia decaocto*). With the Eurasian Collared dove's adaptation, familiar presence, and proliferation in urban environments post-introduction in Algeria, our interest was piqued by the immune system of this species. Consequently, our research aimed to:

- Analyse the hematological indices of Eurasian Collared dove nestlings, tracking the complete blood cell count's progression with age.
- Investigate the immune status of the Eurasian Collared dove within the urban milieu of Annaba.
- Examine the developmental changes in the bursa of Fabricius.
- Conduct a histological examination to discern the variations between juveniles and adults.

Martial and methods

Study site

The study was undertaken in Annaba, Algeria's fourth-largest city (Belabed et al., 2015), positioned approximately 100 km west of the Tunisian border at a latitude of 36° 54′ 15″ North, 7° 45′ 07″ East (Fig. 1).

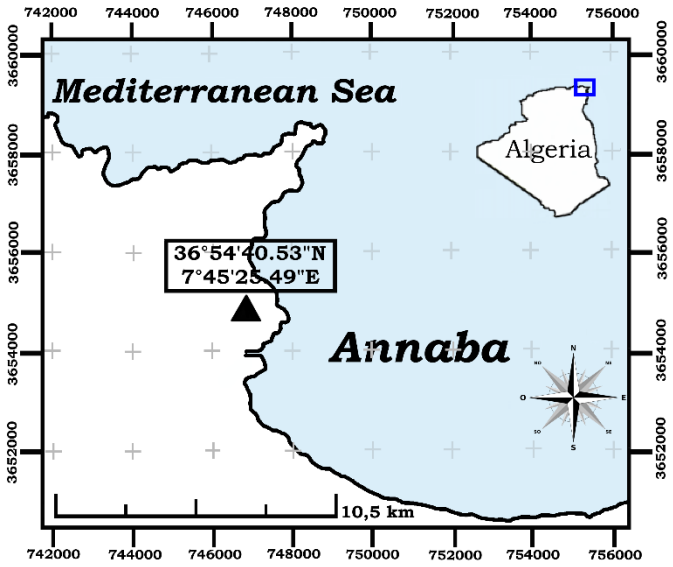


Figure 1. The study site map, the city of Annaba

Biological Model

The Eurasian Collared dove, a recent addition to Algeria's avifauna, has become a prevalent bird in Annaba's urban and suburban regions (Northeast) since its introduction in 1994. As a member of the Columbidae family, it is also known as the Eurasian dove, with its binomial nomenclature being *Streptopelia decaocto* (Frivaldszky, 1838) (Belabed et al., 2013a).

Hematological study

During the breeding season, a systematic search for Eurasian Collared dove nests was conducted, with nestlings being randomly selected for blood sampling post-hatching. The age categories of the selected nestlings were 05 days, 10 days, and 15 days, with a total of five individuals per category (n=5) sampled for blood analysis. Blood smears were prepared immediately after each bird's capture using a droplet of non-heparinized blood from a punctured brachial wing vein using a 23-gauge needle.

For the complete blood cell count, blood smears were prepared following the standard two-slide wedge technique, air-dried, fixed in methanol, and stained with Wright-Giemsa Quick stain. Smears were scanned with a microscope (1000× magnification) and the first 100 leucocytes were differentiated. Obtained cell counts were used for the calculation of the relative proportion of heterophils to lymphocytes (H/L ratios). The cells identified are (Red Blood Cells) erythrocytes (10^6/mm^3 or 1x10^{12}/L) and (White Blood Cells) leukocytes (10^3/mm^3 or 1x10^9/L): Lymphocytes (%); Heterophils (%); Eosinophils (%); Basophils; Monocytes (%) and H/L ratio (Krams et al., 2012).

Immunity status

For the bursa of Fabricius analysis, we investigated five age categories: 05 days, 10 days, 15 days, 18-20 days, and adults, randomly selecting five individuals from each group. These subjects were transported to the laboratory for dissection to excise the bursa. Prior to dissection, each animal was weighed, euthanized following the ethical guidelines approved by the Animal Ethics Commission of UBMA University, Annaba, Algeria, and positioned supine. An incision from the urogenital opening to the neck was made to extract the bursa of Fabricius using fine clamps, which was then weighed on a precision balance (Scaltec, 0.001).

The Bursal Index was calculated for each specimen to assess the immune status of the nestlings, using the following formula:

$$\mathrm{BI} = \frac{Weight\ \ of\ \ the\ bursa\ of\ \ Fabricius}{Body\ \ weight} \times 100$$

Based on the bursal index, the immune status was classified into four categories: BI > 0.20% indicates Excellent immunity; 0.18% < BI < 0.20% signifies Average; 0.15% < BI < 0.18% denotes Mediocre; and BI < 0.15% reflects Poor immune status (Bennett, 2002).

Histological study

For histological examination, each bursa of Fabricius was placed in containers with Bouin's solution. The subsequent procedures included dehydration, blocking, staining, and microscopic observation. These steps facilitated a comprehensive description of the bursa's histological structure, enabling an estimation of the follicle count, which varied with the age, body weight, and bursal weight of each individual.

Statistical analysis

All data are presented as mean ± standard deviation (SD). The statistical significance of the data and correlations was assessed using STATISTICA *StatSoft software* (version 13.2, 2016). A value of $P<0.05$ was considered significant. Principal component analysis (PCA) was performed using XLStat 2019.2.2 (Addinsoft).

Results

Total white blood cell count (WBC)

The analysis across all nestling ages indicated a predominance of lymphocytes (59.489±5.045%) in decreasing order we find heterophils (36.774±5.351%), eosinophils (1.659±2.029%), basophils (1.384±1.670%) and monocytes (0.692±1.258%). For the 05-day nestlings, the results demonstrated the predominance of lymphocytes (57.494±3.431%) in decreasing order we find heterophils (39.027±3.454%), eosinophils (1.872±2.068%), basophils (1.221±1.120%) and monocytes (0.384±0.860%). For the 10-day nestlings, the results show the predominance of lymphocytes (61.182±3.017%) in decreasing order we find heterophils (33.514±3.078%), eosinophils (1.995±1.841%), basophils (1.978±1.806%) and monocytes (1.328±1.839%). For the

15-day nestlings, the results show the predominance of lymphocytes (59.792±7.716%) in decreasing order we find heterophils (37.780±7.611%), eosinophils (1.111±2.484%), basophils (0.952±2.129%) and monocytes (0.363±0.813%). The heterophilic/lymphocyte ratio (H/L ratio) is (0.629±0.145) reaching its maximum on day 5 (0.683±0.099) and its minimum on day 10 (0.550±0.075) (Table 01).

Table 1. Total red blood cells, thrombocytes, and white blood cell count according to age.

	05 Days Mean±SD	10 Days Mean±SD	15 Days Mean±SD	Mean±SD
Erythrocytes (10^6/mm^3)	5.199±0.179	5.818±1.178	5.423±0.202	5.479±0.698
Thrombocytes (10^3/mm^3)	29.6±3.715	31.6±3.781	30.8±2.949	30.667±3.352
Leukocytes (10^3/mm^3)	45.8±5.541	30.4±2.509	50.8±6.058	42.333±10.090
Lymphocytes (%)	57.494±3.431	61.182±3.017	59.792±7.717	59.489±5.045
Heterophils (%)	39.0278±3.454	33.514±3.078	37.781±7.611	36.774±5.351
Eosinophils (%)	1.8724±2.069	1.996±1.842	1.111±2.484	1.659±2.029
Basophils (%)	1.221±1.121	1.978±1.807	0.952±2.129	1.384±1.670
Monocytes (%)	0.385±0.860	1.329±1.839	0.364±0.813	0.692±1.259
H/L ratio	0.683±0.099	0.550±0.075	0.654±0.215	0.629±0.145

The dynamics of changes of white blood cell count (WBC)

The white blood cell count varied with age. Lymphocytes peaked on day ten and were lowest on day five. Conversely, heterophils were highest on day five and lowest on day ten. The counts of other cells (eosinophils, basophils, and monocytes) reached their zenith in the ten-day samples, with the fifteen-day samples showing the lowest values (Fig. 2).

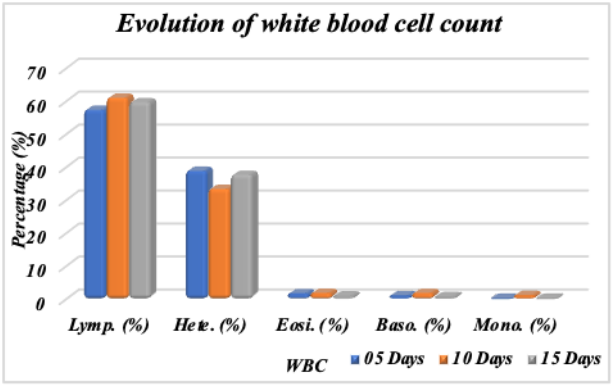


Figure 2. Evolution of white blood cell count (WBC).

The dynamics of changes in the count of red blood cells (RBC)

The blood composition reflects the health status of the nestlings. On the 5th day, red blood cells (RBCs) were less abundant, contrasting with the higher count of white blood cells (WBCs). However, by the 10th day, there was a notable surge in erythrocytes and a reduction in leukocytes.

Approaching the fledging stage on the 15th day, an inverse trend was observed: an upsurge in WBCs and a decline in RBCs, leading to fledglings exhibiting signs of anemia (Fig. 3).

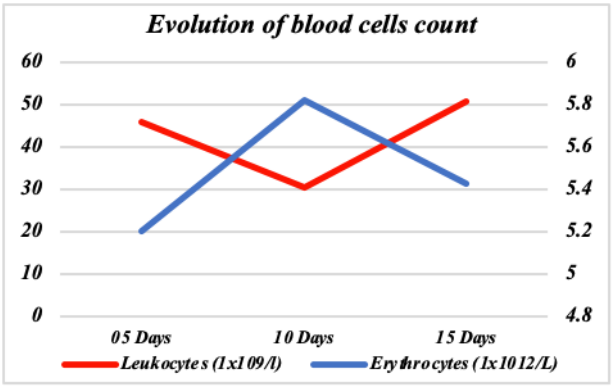


Figure 3. Evolution of the count of red blood cells (RBC).

Evolution of the body weight

Prior to dissection, the nestlings' weights were recorded, with the findings presented in Table 2 and Fig. 4. There was a consistent increase in the average weight among the five age groups, indicating growth progression.

Table 2. Body weight of the different individuals according to age (g).

	5 Days	10 Days	15 Days	18-20 Days	Adults
N 01	52.04	85.00	113.20	98.00	183.00
N 02	54.00	88.00	112.00	96.20	181.40
N 03	45.93	76.57	104.25	91.30	184.00
N 04	45.00	78.00	98.00	96.20	187.00
N 05	42.57	82.58	99.40	97.12	192.00
Mean±SD	46.59±5.42	82.36±3.96	104.81±5.86	95.64±2.15	186.07±7.82

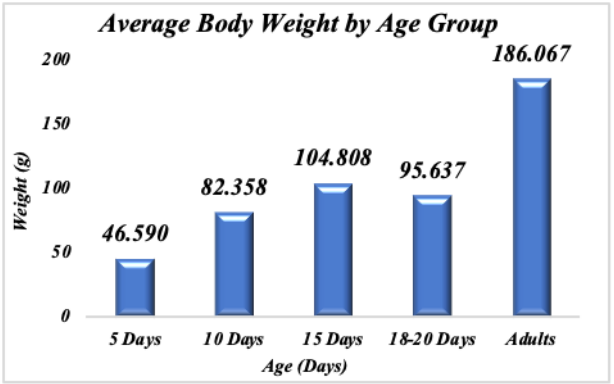


Figure 4. Evolution of the body weight according to age.

Post-dissection, the weights of the Fabricius bursa were documented, with the outcomes illustrated in Fig. 5. A steady reduction in the average weight of the bursa across the five age groups was noted, suggesting developmental changes.

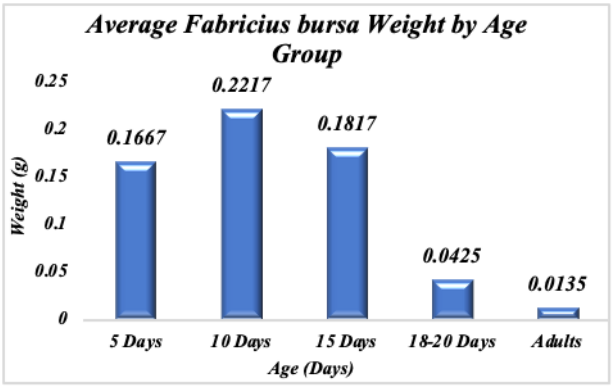


Figure 5. Evolution of the Fabricius bursa weight according to age.

Relationship between the weight of the individual and the weight of the bursa of Fabricius according to age

As depicted in Fig. 6, during the initial age range (0 to 10 days), there was a correlation between the average weight of the nestlings and the weight of the bursa of Fabricius, attributed to rapid developmental changes. Beyond the 10th day, a gradual increase in the weight of the nestlings was observed, whereas the bursa weight exhibited a decline.

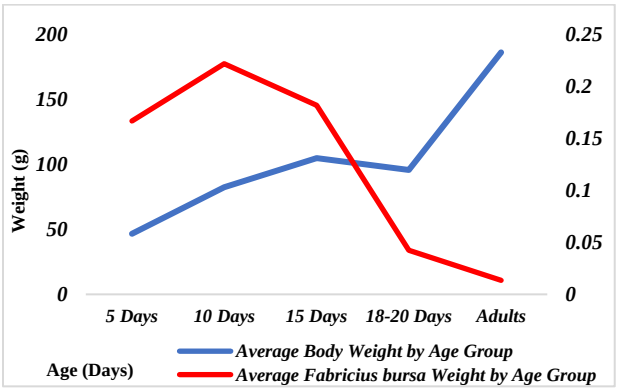


Figure 6. Evolution of the individual and the bursa of Fabricius weights.

Evolution of the bursal index according to age

As illustrated in Fig. 7, the nestlings' immune status is excellent at birth and maintains this level up to 10 days, as indicated by the bursal index. However, after reaching this age threshold, there is a noticeable deterioration in immune status, transitioning from "excellent" to "mediocre" by approximately 15 days, and further declining to "poor" status around 20 days, a trend that persists into adulthood.

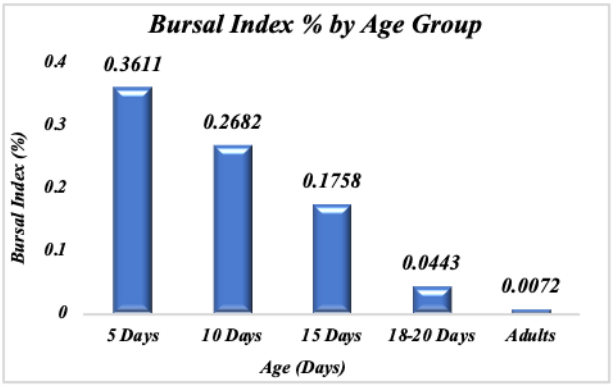


Figure 7. Evolution of the immune status by age group.

A highly significant and negative correlation (***) was observed between the average weight of the individuals and the bursal index (%) (r=-0.7909; p=0.0000).

Relationship between the weight of the bursa of Fabricius and the number of follicles according to age

The analysis revealed a continuous increase in the weight of the bursa from 05 days of age, peaking at 10 days with a maximum weight 0.221±0.071 g. Concurrently, the number of bursal follicles reached its zenith at 10 days, totalling 400.00±23.64 follicles. Beyond 10 days, a marked decrease was noted: the bursa's weight dropped to 0.18±0.052 g at 15 days and further to 0.042±0.007 g by 18-20 days. Simultaneously, the follicle count diminished to 218.00±28.50 at 18-20 days, eventually dwindling to 46.00±8.32 in adults. This trend underscores a clear age-dependent relationship between the bursa of Fabricius's weight and its follicle count (Fig.8).

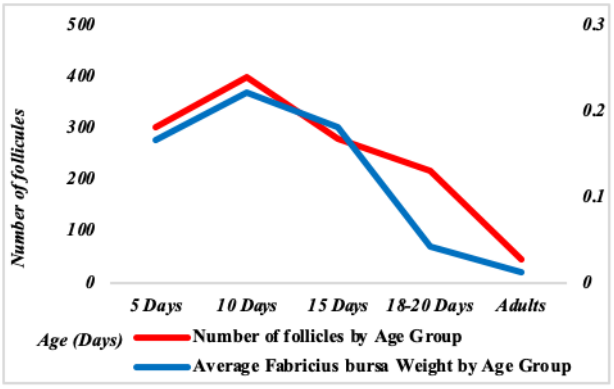


Figure 8. The Relationship between the weight of the bursa of Fabricius and the number of follicles according to age.

The correlation between the bursa of Fabricius weight and the number of follicles is positively strong and highly significant (***) (r=0.8806; p=0.000).

PCA of the health status according to age

The health status was analyzed using Principal Component Analysis (PCA) based on age, utilizing twelve variables: (Nb of follicles, Average Body Weight, Average Fabricius bursa Weight, Bursal Index (%), Erythrocytes (10^6/mm^3), Leukocytes (10^3/mm^3), Lymphocytes (%), Heterophils (%), Eosinophils (%), Basophils (%), Monocytes (%), H/L ratio,). The employed software generated vectors on the factorial axes, indicating the influence and intensity of the factors on the dataset.
The first two factorial axes accounted for 30.84% and 22.02% of the variance, summing to 52.86% of the total variance. Therefore, only these two axes were considered for subsequent analysis. The size of the dot-area in the PCA plot corresponds to the magnitude of the variables' values.
When projected onto the F1xF2 plane, the data points clustered into five distinct age groups (Fig. 9). In red, is the 05 days group, in purple is the 10 days group, in yellow is the 15 days group, in orange is the 18-20 days group and, in green is the adult group.

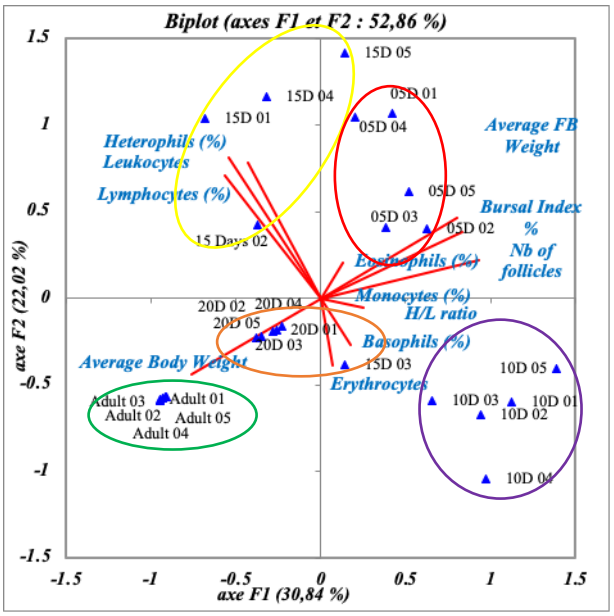
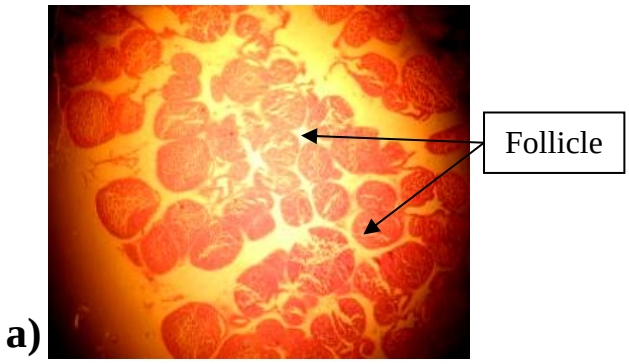
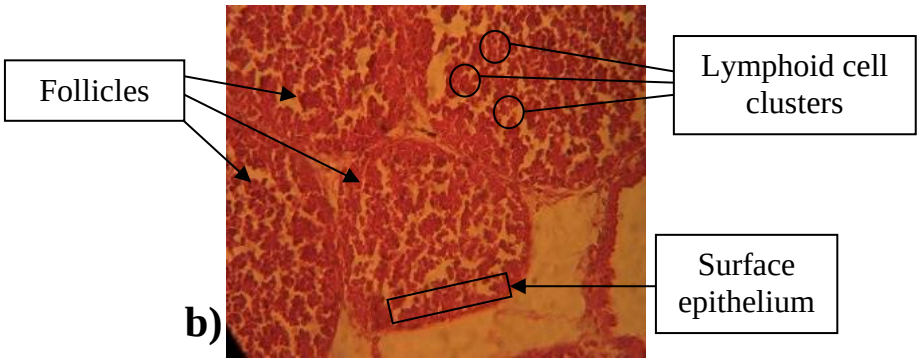
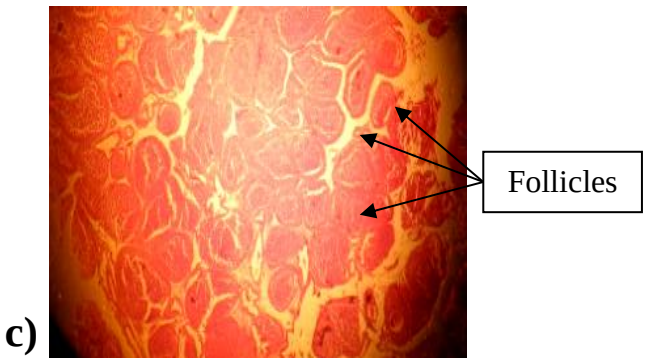


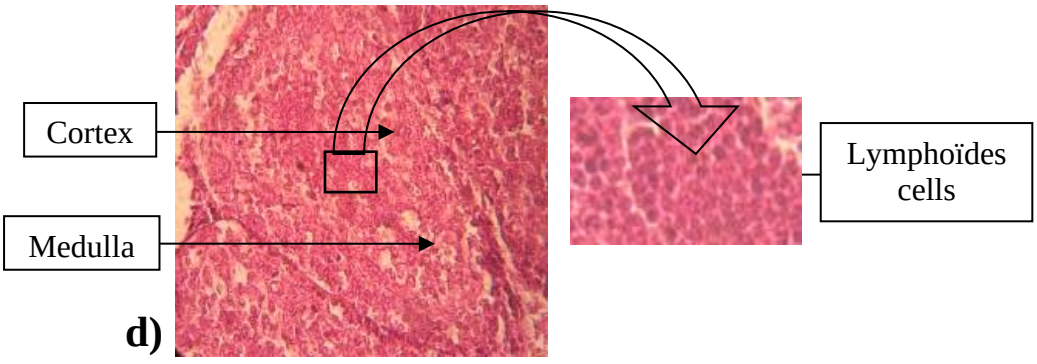
Figure 9. Projection on the F1xF2 plan of PCA.

Microscopic Study


Follicle
a)


Follicles
Lymphoid cell clusters
Surface epithelium
b)


Follicles
c)


Cortex
Medulla
Lymphoïdes cells
d)

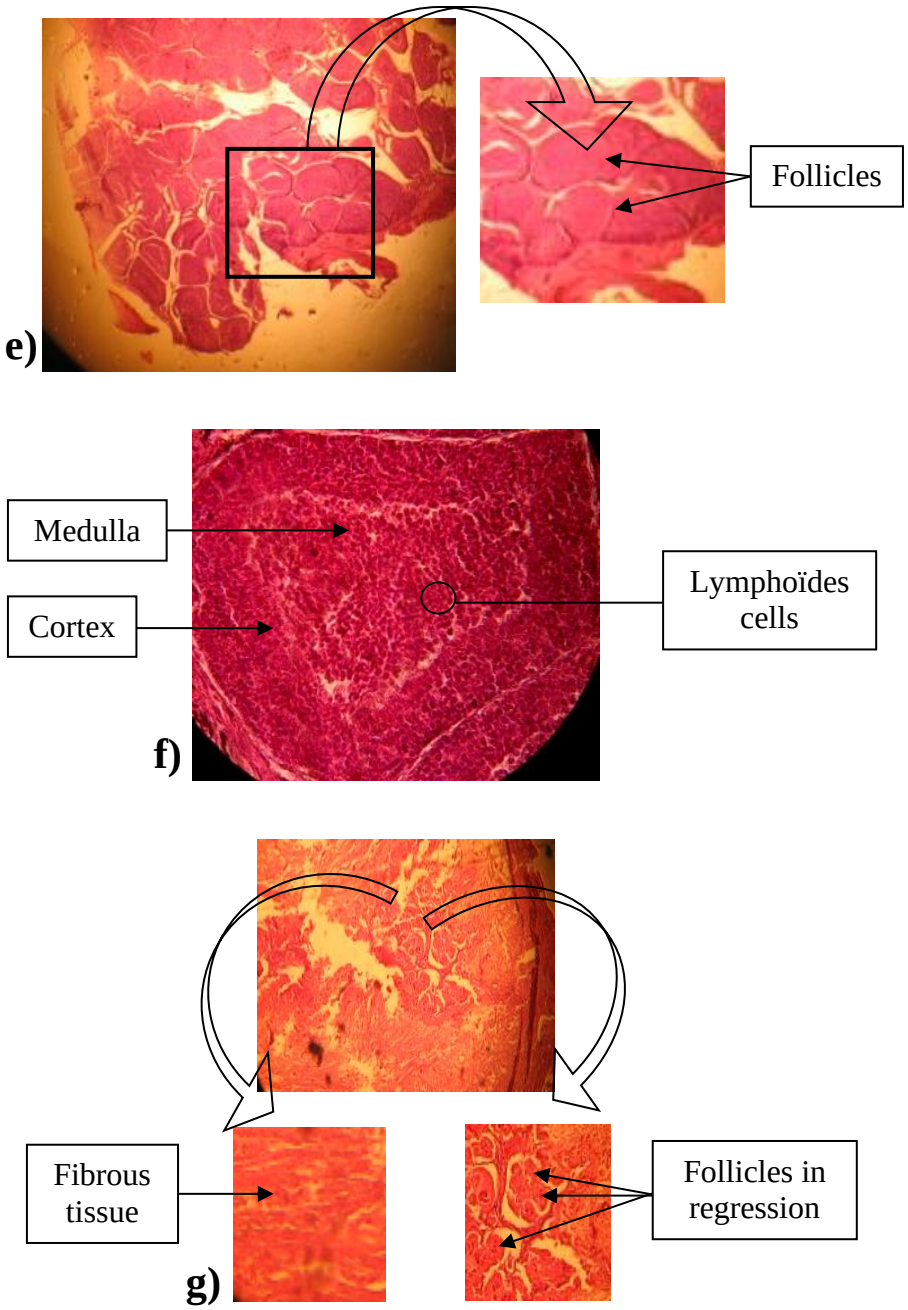


Figure 10. Sections of the bursa of Fabricius of the Eurasian Collared dove under the microscope.

a) Section of the bursa of Fabricius of 05 days age Eurasian Collared dove under the microscope (40× magnification).
b) Section of the bursa of Fabricius of 05 days age Eurasian Collared dove under the microscope (60× magnification).
c) Section of the bursa of Fabricius of 10 days age Eurasian Collared dove under the microscope (40× Magnification).

d) A follicle of the bursa of Fabricius of a 10 days Eurasian Collared dove under the microscope (60× Magnification).
e) Section of the bursa of Fabricius of 15 days age Eurasian Collared dove under the microscope (40× magnification).
f) A follicle of the bursa of Fabricius of a nestling at the age of 20 days Eurasian Collared dove under the microscope (60× magnification).
g) Section of the bursa of Fabricius of an adult Eurasian Collared dove under the microscope (60× magnification).

Histology of the bursa of Fabricius at the age of 05 days

Histological examination reveals that the bursa is encased by a distinct surface epithelium and contains numerous bursal follicles, which dominate the space within each fold. These follicles, polyhedral in shape, are separated by a slender layer of connective tissue. Typically, each follicle comprises two regions: the cortex and the medulla. However, due to the sparse distribution of lymphocytes in 5 days old bursal follicles, distinguishing between these two regions is challenging (Fig. 10a and Fig. 10b).

Histology of the bursa of Fabricius at the age of 10 days

Comparative analysis with earlier sections shows a notable increase in both follicle number and lymphocyte density in the bursa. This denser cellular arrangement facilitates the identification of the cortical and medullary regions within the follicles, with the distinction becoming more apparent due to reduced inter-follicular space (Fig. 10c and Fig. 10d).

Histology of the bursa of Fabricius at the age of 15 days

The follicle count and bursa size are reduced, but each follicle is densely populated with lymphocytes, distinctly showcasing the cortex and medulla. These sections are demarcated by a basal membrane, highlighted by a capillary network, with lymphoepithelial follicles adjacent to the surface epithelium (Fig. 10e).

Histology of the bursa of Fabricius at the age of 18-20 days

The histological analysis of the bursa from an 18-20-day-old nestling mirrors the findings at 15 days of age, indicating a developmental consistency in the bursal structure (Fig. 10f).

Histology of the bursa of Fabricius at Adult age

In adult Eurasian Collared doves, the regression of the bursa is pronounced, characterized by widespread organic fibrosis and densely packed follicles, with the entire space becoming saturated with fibrous tissue (Fig. 10g).

Discussion

Health significantly influences body condition and vitality, which, in turn, affects an individual's fitness. Therefore, adopting precise and reliable methods to estimate health status is paramount for ecological and evolutionary studies (Vinkler et al., 2010). Organisms, including birds, face continuous threats from parasites and pathogens, necessitating robust defense mechanisms. The avian immune system, comprising both innate and adaptive components with cellular and humoral elements, is a critical defense arsenal against such threats (Krams et al., 2012). Birds employ hematological parameters and immune functions to gauge individual health within their populations, where cell counts serve as biomarkers of immune responsiveness. Specific immunity, triggered by antigen recognition, leads to an increased lymphocyte count, whereas non-specific immunity provides a broad-spectrum defense (Campbell, 2015). The prevalent approach for health evaluation in avian species is the basic hematological survey (Vinkler et al., 2010). Assessing hematological and biochemical parameters furnishes a comprehensive method to appraise the body condition of free-ranging birds. The primary focus of these surveys is to ascertain the cellular makeup of the blood, which includes lymphocytes, monocytes, and granulocytes, the latter subdivided into heterophils, eosinophils, and basophils (Campbell, 2015). Cellular proportion norms can vary across species (Campbell, 2015) and between wild and captive birds (Ewenson et al. 2001). Typically, lymphocytes and heterophils are the primary cells counted in birds for reliable interindividual comparisons. The lymphocyte-to-heterophil (H/L) ratio is widely recognized for its reliability in indicating stress levels (El Lethey et al., 2003; Davis, 2008) and infection status in various diseases (Fokidis et al., 2008; Norte et al. 2009), making it a favored measure of health status due to its simplicity, repeatability, and ease of determination (Vinkler et al., 2010). Avian heterophils are functionally analogous to mammalian neutrophilic granulocytes, exhibiting potent phagocytic activity and playing a pivotal role in the initial inflammatory response (Genovese et al., 2013; Hofmann et al., 2020). In assessing the health of our avian subjects, we focused on parameters that reflect the health and immune status, including blood cell counts, body condition, and the state of the bursa of Fabricius, to understand the impact of age on these indicators.

Remarkably, the red-to-white blood cell ratio in nestlings showed a mean value of 114.718±13.0340 at 5 days, 190.755±30.721 at 10 days, 108.3267±17.036 at 15 days, with an overall average of 137.933±42.146. This range aligns with established avian norms of 70 to 200 (Beaumont & Cassier, 1987). These findings concur with those reported for the Eurasian Collared dove *(Streptopelia decaocto*), where a ratio of 151.428 was observed (Lashev, 2009), and subsequent studies yielded ratios of 181.791 in 2014, 193.572 in 2017, and 170.41 in 2019 for the same species (Sinan, 2014; 2017; 2019). An analysis of our data revealed a trend of relative anemia in nestlings before the flight, characterized by elevated white blood cell counts (from 5.818±1.178 $10^6/mm^3$ at 10 Days to 5.423±0.202 $10^6/mm^3$ at 15 Days) followed by a major increase in the level of white blood cells from day 10 to day 15 (30.4±2.509 $10^3/mm^3$ at 10 Days to 50.8±6.058 $10^3/mm^3$ at 15 Days) that would be indicators of an immune response. This decline in red blood cells could be attributed to parasitic infections, which can rapidly affect hematocrit levels (Boross et al., 2012). Our results for the RBC ($\times10^6/mm^3$) and the WBC ($\times10^3/mm^3$) of the Eurasian Collared dove, are similar than those of (Lashev, 2009) (RBC $5.3\times10^6/mm^3$; WBC $35\times10^3/mm^3$) and, (Sinan, 2014;

2017; 2019) (RBC 4.572±3.121×10^6/mm^3; WBC 25.152±1.159×10^3/mm^3), (RBC 4.502±0.206×10^6/mm^3; WBC 23.26±1.272×10^3/mm^3), (RBC 2.885±1.498×10^6/mm^3; WBC 16.93±7.699×10^3/mm^3) for Eurasian Collared dove (*Streptopelia decaocto*).

Erythrocyte indices, particularly hemoglobin concentration and hematocrit, are increasingly recognized as vital indicators of avian health (Lill et al., 2013). The hematocrit value typically rises with age due to enhanced erythropoiesis, resulting in higher hematocrit levels in adults compared to nestlings or juveniles (Fair et al., 2007). Furthermore, the impact of parasitism is more pronounced in nestlings than in adults, attributed to their underdeveloped immune systems (Dunn et al., 2017). The development of immune defenses progresses with age, rendering younger birds less capable of mounting a robust immune response compared to their older counterparts (MacColl et al., 2017).

In our study, lymphocytes emerged as the predominant leukocytes in the blood smears. We observed an average lymphocyte percentage of 57.494±3.431% in 5-day-old nestlings, 61.182±3.017% in 10-day-old nestlings, 59.792±7.717% in 15-day-old nestlings, with an overall average of 59.489±5.045%. Elevated lymphocyte levels, similar to those noted in avian malaria infections, suggest a significant immunological response (Figuerola et al., 1999). Lymphopenia, indicative of corticosteroid excess, often leads to a combined state of leukopenia and lymphopenia, particularly as an early response in a corticosteroid-induced leukogram, which could imply viral infections (Campbell, 2015). Leukocytosis and heterophilia are frequently associated with a broad range of infectious agents (i.e., bacteria, fungi, *Chlamydophila*, viruses, *Mycoplasma*, and parasites) and noninfectious etiologies (i.e., traumatic injury, toxicities, stress). In cases of inflammation, these conditions are typically accompanied by an increase in heterophils, underscoring their role in the immune response (Campbell, 2015). Heterophils, crucial in the avian immune defense, act as the primary responders to invasive pathogens, paralleling the function of mammalian neutrophils. Their engagement is essential for initiating and driving the immune system towards effectively combating pathogens (Genovese et al., 2013). Our findings show a heightened presence of heterophils, possibly indicative of the immune system's reaction to vector binding, which frequently triggers heterophil-involved inflammatory responses. The surge in circulating heterophils is generally seen as a marker of inflammation and stress (Campbell, 2015; Minias, 2019), with our data showing average heterophil percentages of 39.028±3.454% in 5-day-old nestlings, 33.514±3.078% in 10-day-old nestlings, and 37.781±7.611% in 15-day-old nestlings, leading to an aggregate average of 36.774±5.351%.

The key cellular components of the innate immune response, particularly in the wake of inflammation or parasitic invasion, include eosinophils, heterophils, and monocytes. This innate immunity forms the initial barricade against infection, often neutralizing the threat before the activation of the host's adaptive immune system (Koonpaew et al., 2019). Our results align with previous findings, such as those by (Lashev, 2009) (Lymp. 53.5%; Heter. 41.7%) and (Sinan, 2017) (Lymp. 54.195±2.797%; Heter. 42.937±2.906%), along with (Sinan, 2019) (Lymp. 30.042±14.473%; Heter. 38.95±6.746%).

Eosinophils represent the second type of acidophilic granulocytes found in avian blood, typically less prevalent than heterophils across many species (Clark et al., 2009). Interpreting eosinophilia in birds is challenging, given the unclear role of avian eosinophils. Although termed "eosinophils," their behavior may differ from that of their mammalian counterparts. Despite limited knowledge of avian eosinophil function, peripheral eosinophilia in birds is generally associated with responses to parasitic infections or foreign antigens, indicating a hypersensitivity reaction (Campbell, 2015). The function of eosinophils in birds remains partially understood; nevertheless, they are believed to play a crucial role in controlling primarily ectoparasites (Meeusen & Balic, 2000). Elevated levels of eosinophils and heterophils are also noted in immune-compromised individuals (Campbell, 2015). Heterophils, as rapid-response leukocytes, engage in microbial defense, often through oxidative mechanisms, upon initial exposure (Schat et al., 2013). Historically viewed as terminal cells in the defense against parasitic infections and immunopathology in hypersensitivity diseases, recent research has redefined eosinophils as multifaceted leukocytes that contribute to tissue homeostasis, the modulation of adaptive immune responses, and innate defenses against certain pathogens (Kita, 2011). Their diverse functions are particularly significant in combating helminth infections and other extracellular parasites (Schat et al., 2013; Kita, 2011; Ruhs et al., 2020).

Plasma glucocorticoids are well-established and frequently utilized biomarkers for stress in vertebrates (Aerts, 2018). The act of capturing and handling birds for blood collection is recognized as a significant stressor, triggering rapid alterations in glucocorticoid levels (Johnstone et al., 2012). Biomedical and ecological research across various taxonomic groups has demonstrated that stress in animals leads to an increased ratio of specific white blood cells (neutrophils in mammals, amphibians, and fish; heterophils in birds and reptiles) to lymphocytes, indicating a heightened stress response. This ratio, known as the N/L or H/L ratio, is particularly responsive to diverse stressors in nestling birds, with notable sensitivity to food availability (Müller et al., 2011; Bańbura et al., 2013).

The heterophils/lymphocytes (H/L) ratio in nestlings exhibited noticeable variations, registering the highest at 0.683±0.099 in 5-day-old nestlings, and decreasing to 0.550±0.075 in 10-day-old nestlings. The computed average ratio stood at 0.629±0.145, indicating a relatively high H/L ratio. Comparable ratios are observed in similar avian species : Eurasian Collared doves (*Streptopelia decaocto*) 0.76±0.023 (Lashev, 2009); Domestic pigeon (*Columba livia*) 0.673±0.015 (Lashev, 2009); Pink-headed Fruit Pigeon (*Ptilinopus porphyreus*) 0.52 (Schultz, 2003); White-winged Dove (*Zenaida asiatica*) 0.42 (Small et al., 2005). But different compared to other species: African Collared dove (*Streptopelia roseogrisea*) 1.05±0.0381 (Lashev, 2009); Nicobar Pigeon (*Caloenas nicobarica*) 1.39 (Peinado et al., 1992); Pheasant Pigeon (*Otidiphaps nobilis*) 1.27 (Peinado et al., 1992); Southern Crowned pigeon (*Goura scheepmakeri*) 1.51 (Peinado et al., 1992); Victoria Crowned pigeon (*Goura victoria*) 1.18 (Peinado et al., 1992); Common crowned pigeon (*Goura cristata*) 2.18 (Peinado et al., 1992).

While the H/L ratio serves as a reliable indicator for various stress types in birds (Minias, 2019), interpreting these values can be complex. The H/L ratio is an index dependent on changes in two

cell kinds engaged in immune response; heterophils are phagocytes involved in the first line of innate defense against infections, while lymphocytes are involved in the highly specific acquired defense (Davis, 2008; Campbell, 2015). Since different cellular components of the immune system may develop along different trajectories with the growth of an organism, age must be included in all comparisons in the case of studying the H/L ratio of nestlings (Johnstone et al., 2012; Bańbura et al., 2013). The discrepancy between our findings and those of other studies could be attributed to the differing ages of the nestlings examined; unlike studies focusing on adult birds, we observed varying H/L ratios across different ages and species. This suggests that H/L ratio alterations might be more rapid and species-specific than previously understood (O'Dell et al., 2014). In studies of free-living Eurasian kestrel nestlings (*Falco tinnunculus*), it was confirmed that the H/L ratio increased in response to several natural stressors. These include competitive disadvantage due to later hatching, low body fat reserves, ectoparasite infestation, post-rain challenges affecting food delivery by adults, and progressive seasonal environmental deterioration (Müller et al., 2011). The H/L ratio not only escalates in reaction to mild stressors but also in the face of severe, life-threatening conditions such as starvation. Nevertheless, it is imperative to acknowledge that H/L levels can be influenced by disease and infection, potentially obscuring the true reflection of stress in response to external factors (O'Dell et al., 2014). Elevated H/L ratios can signal human-induced alterations in the structure of breeding habitats, highlighting a significant ecological impact (Bańbura et al., 2013). Another line of investigation posits that filarial parasites may compromise a host's capacity to generate immune cells in response to antigens (Chatterjee et al., 2015) while concurrently elevating inflammatory neutrophils. Consequently, a microfilaria infection could lead to heightened H/L ratios if similar immune modulation occurs in birds, characterized by a reduction in lymphocytes and an increase in heterophils. These hematological changes potentially impair the host's ability to manage pathogens through antigen recognition (Clark et al., 2016). Furthermore, inflammation might influence the H/L ratio by both augmenting heterophil concentration through enhanced granulocytopoiesis and diminishing lymphocyte concentration due to cytokine-induced rises in corticosterone levels (Clark, 2015). Given these complexities, interpreting these hematological indices necessitates caution (Norris & Evans, 2000). An elevated lymphocyte count in parasitized individuals might indicate a protective response against the parasite, yet it could also signify individuals debilitated by other factors, thus hindering their capability to combat the parasites effectively.

This dichotomy suggests that if immune indices are indicative of immunocompetence, individuals with higher values would typically exhibit a lower parasite burden. Conversely, if immune indices are reflective of ongoing infections and the current activation level of the immune system, individuals with higher values may exhibit more severe parasitization (Biard et al., 2015).

The immune system's primary function is to forge a protective barrier against deleterious agents such as pathogens and toxins, all while minimizing collateral damage to the host (Male et al., 2012). The innate immune system, a fundamental component of host defense, encompasses a variety of immune cells and chemical defenses, offering a nonspecific defense mechanism that activates within minutes to hours following an infection and exists across all living organisms

(Helin, 2020). The adaptive immune system stands as a pivotal branch, initiated through signals from the innate immune system and the interaction of antigens with receptors on antigen-presenting cells (APCs), notably the lymphocytes within the adaptive system. The activation process for the adaptive immune system is relatively slower upon initial exposure to a novel pathogen, but once triggered, it orchestrates a response that is notably more precise and specific compared to the innate immune response (Helin, 2020). Birds exhibit a distinct immune configuration, with the thymus and the bursa of Fabricius serving as the primary immune organs (Ifrah et al., 2017; Schat et al., 2013; Helin, 2020).

Unique to birds and absent in mammals, the bursa of Fabricius is an epithelial and lymphoid structure located near the cloaca. This organ plays a crucial role in mediating humoral immunity, particularly in the production of antibodies, and typically regresses as the bird reaches sexual maturity (Schat et al., 2013; Khenenou et al., 2012). The size of the bursa of Fabricius can be indicative of the immune investment in birds, with a larger size suggesting a more substantial investment in immune function. This organ is predominantly present in juvenile birds and is integral during the development of the B cell repertoire (Stenkewitz et al., 2015). Described as a blind, lymphoepithelial diverticulum of ectodermal origin, the bursa of Fabricius is situated in the dorsal proctodeum of the avian cloaca (Nagy & Oláh, 2010). Acting as both a primary and secondary lymphoid organ, it facilitates the diversification and maturation of B cells, which are crucial for responding to dietary and environmental antigens encountered within its lumen. Exclusive to birds, the cloacal bursa's functional counterparts in mammals are located in different organs (Løken et al., 2019; Bódi et al., 2019). The organ's structure, typically round or oval in chickens and elongated in ducks, features 10 to 15 folds that line its lumen, encompassing 8,000 to 12,000 follicles, thus generating a vast diversity of antibodies. As a primary lymphoid organ, the bursa of Fabricius is evident upon hatching in precocial birds, with the involution of bursal tissue varying among species. This involution is contingent upon factors such as age, sexual maturation, or commencement of egg production (Mešťanová & Varga, 2016). Prior to their regression, the thymus and the bursa of Fabricius cede control to the secondary immune organs, which remain functional throughout the bird's life. The mucosal architecture of the bursa of Fabricius can be delineated into two distinct configurations. The first, known as the "plicae type," features longitudinal plicae within the bursa of Fabricius's cavity, where the mucosal epithelium of these plicae initiates the formation of epithelial buds. These buds serve as the central point for the growth of lymph follicles. Contrasting this is the "tubulous type," where the tubular structure branches extensively from the main duct inside the bursa of Fabricius. In this arrangement, the epithelial bud emerges on one side of the tubulus, expanding to develop lymph follicles, which are infrequently established on the main duct's wall (Schat et al., 2013).

In the initial phases of the bursa of Fabricius formation and during its involution, the distinction between these two types is pronounced. However, this clarity diminishes in the mature stages due to the mucous membrane's distorted structure, a consequence of the significant expansion of the lymph follicles. This complexity has led to the differences between the two types being somewhat overlooked in the scientific discourse (Schat et al., 2013).

The immune system bifurcates into two primary responses:

- The specific immune response encompasses humoral and cellular immunity, necessitating an antigenic stimulus to incite a targeted immune reaction, resulting in the production of specific antibodies for each antigen.
- The non-specific immune response operates indiscriminately against a broad range of antigens.

B cell lymphocytes, originating from the bursa of Fabricius, are pivotal in humoral immunity, while T lymphocyte cells, derived from the thymus, play a crucial role in cellular immunity. Macrophages, heterophils, and thrombocytes constitute the core elements of the non-specific immune system.

Research has delved into the invasive tendencies of the Eurasian Collared dove, alongside studies on adaptive mechanisms. The findings from our study indicate that the average weight of the sampled individuals escalates with age, a phenomenon attributed to the parental commitment to nourish the brood, thereby optimizing their prospects for future recruitment.

A noteworthy positive correlation was observed between the nestlings' weight and the bursa of Fabricius during the initial 10-day period. At the point of hatching, the nestlings' bursa of Fabricius is already well-developed, exhibiting rapid growth thereafter. The growth rate of this organ is markedly variable across different species and is also modulated by factors such as gender and breeding practices (Mešťanová & Varga, 2016).

This leads to the inference that the weight and dimensions of the bursa of Fabricius are proportionally related to the age and weight of the nestling (Alloui et al., 2020).

The findings from our study highlight that the bursa of Fabricius in the observed model attains its maximum volume at around 10 days of age. During this critical growth phase, a physiological regression is noted, attributed to a significant reduction in the size of this organ in nestlings aged between 10 and 18 days, with it being nearly absent in adult specimens.

In avian species where the developmental trajectory of the bursa has been meticulously charted, an enlargement of the bursa is typically observed soon after hatching, culminating in a peak size that is determined by the bird's race or species. Following this peak, there is a linear diminishment in size, with involution generally completed by the end of the first year of life, again contingent on the race or species of the bird. In adult birds, the bursa is markedly diminished in size or completely absent, occasionally persisting as a small sac located on the dorsal side of the cloaca (Schat et al., 2013).

Utilizing the method delineated in (Bennett, 2002), the bursal index calculated for this study indicates that our biological model exhibits an immune deficiency that intensifies with age. Initially, the nestling presents with an "Excellent" immune status, which, as time progresses, deteriorates through "Mediocre" levels, ultimately reaching a "Bad" immune condition in adulthood. However, this decline in bursal size and function should not be interpreted as an indicator of poor health in the Eurasian Collared dove; it reflects the natural dispersal of immune function to other vital organs as birds age.

In our research, we quantified the follicle count relative to the age of the subjects. The lymph follicles in the bursa of Fabricius predominantly displayed an invaginated structure. Both in the

plicae and tubulous types, the follicle comprises lymphoepithelial and lymphoreticular components, with the detailed structure of these follicles not being the focus of our discussion (Schat et al., 2013).

Our analysis revealed a significant positive correlation between the weight of the bursa of Fabricius and the follicle count. Conversely, a significant negative correlation was found between the weight of the individual birds and their follicle count, suggesting that as the bursa regresses and atrophies, the number of follicles decreases accordingly.

Comparatively, the average follicle count in 5-day-old Dove nestlings was found to be around 400, in stark contrast to the 15,000 follicles typically observed in domestic broiler species, illustrating significant species-specific variations in bursal structure and function.

As the nestlings age, there is a gradual increase in the number of lymphoid cells within the follicles, leading to a progressive cellular fill. Consequently, the distinction between the medulla and cortex, which was previously evident, becomes obscured in the nestlings between 5 to 10 days of age. This obscurity is attributed to the proliferation of immune cells as the nestling begins to encounter various factors that challenge its immunity.

Environmental aggressions, such as stress, poor hygiene, vaccination, and diseases, significantly impact the histo-anatomical and physiological development of the bursa of Fabricius. These factors can induce immunodepression in some birds, reflecting the sensitivity of the bursa to external conditions (van den Berg, et al., 2000; Al-Tememy et al., 2011).

In adult birds, the bursa of Fabricius exhibits signs of physiological involution, marked histologically by the gradual reduction of lymphoid cells in the follicles, leading to fibrosis and a substantial decrease in organ volume.

When comparing the age-related structural changes in the bursa of Fabricius between our model bird and *Columba livia*, a species from the same family, reference is made to the findings of (Ciriaco et al., 1989). These authors have documented that the involution process in the bursa begins at the epithelium, extending to the follicular medulla and other organ structures. This process involves mucoid degeneration followed by significant fatty degeneration. They identified three primary phases in the organ's regression: early (from 90 to 150 days), late (up to 165 days), and residual (from 165 to 180 days) involution stages.

The early involution stage is typified by the presence of epithelial cells adorned with long microvilli, which contain granules at the apical pole, and a relatively normal bursal structure. However, the epithelium shows a progressive increase in mucin-producing goblet cells density, alongside notable enlargements in intercellular spaces and the formation of voluminous cysts. Changes in the follicles become evident, marked by expanded intercellular spaces and an enhanced connective framework.

During the late involution stage, the epithelium exhibits a gradual flattening, with an increase in the size and number of microvilli. Secretory epithelial cells diminish in size, yet the quantity of secretory granules escalates. Additionally, the period sees an augmentation in intercellular spaces, emergence of blood vessels, presence of numerous small lymphocytes in the lumen, and fat cells laden with multiple lipid droplets.

The cysts proliferate, encircled by epithelial cells, leading to a reduction in follicle count and the blurring of distinctions between cortex and medulla. Concurrently, connective tissue progressively supplants the follicles, accompanied by the incursion of large blood vessels.

By the residual stage, lymphoepithelial structures are supplanted by connective tissue, which incorporates blood vessels and large fat droplets, with the presence of smooth muscle cells also noted. Aging impacts the secretory cells, manifesting in a progressive decline in the number and size of secretory granules, the disintegration of break-down bodies, and a reduction in the length of typical cytoplasmic processes (Gallego et al., 1996; Ciriaco et al., 2003).

The histological examination of the specified lymphoid organ, the bursa of Fabricius, revealed no histopathological lesions or abnormalities. This sac-like structure, positioned on the dorsal wall of the cloaca's proctodeum, is characterized by a slightly obscured interior due to the presence of numerous well-developed mucosal folds (Schat et al., 2013).

Blood vessels that nourish the organ are nestled within the muscular tissue at the base of the mucous membrane's folds, extending into the chorion. The mucous membrane, notably the thickest segment of the bursa's wall, is intricately composed of:

- The frame of connective tissue and the follicle;
- The epithelial surface.

In each mucosal fold, the follicles, prominently polyhedral in shape, occupy the largest area and are demarcated from each other by a slender layer of connective tissue.

Each follicle is characterized by two distinct regions: the cortex and the medulla, which are delineated by a basal membrane. This membrane's existence is highlighted by a surrounding network of blood capillaries, emphasizing its significance in the organ's structure.

Each follicle is bifurcated into two segments, the cortex and the medulla, with this division underscored by a basal membrane. This membrane's presence is demarcated by a network of blood capillaries. In our study, we evaluated twelve parameters to ascertain the health status across different age groups: number of follicles, average body weight, average bursa of Fabricius weight, bursal index percentage, erythrocytes, leukocytes (1x109/l), lymphocytes (%), heterophils (%), eosinophils (%), basophils (%), monocytes (%), and the H/L ratio. Principal Component Analysis (PCA) delineated five distinct clusters, each representing a specific age group. The 5-day group was marked by the weight of the bursa of Fabricius, bursal index, and eosinophil levels; the 10-day group by erythrocytes and basophils; the 15-day group by leukocytes, lymphocytes, and heterophils; and the 20-day and adult groups were notable for body weight distinctions.

Conclusion

The primary objective of our investigation was to deepen the understanding of the immunity and health status of the Eurasian Collared dove nestlings within urban ecosystems, particularly about the species' invasion dynamics in our region. Our findings aimed to elucidate the impact of the immune system on various morphological and hematological parameters, revealing no detrimental effects on the nestlings' body condition, thereby suggesting a successful adaptation to the environmental pressures of their new habitats. Delving into the cellular-level immunity, our focus

expanded to the overall immune status of this species. Through our analyses, the bursal index was instrumental in illustrating that the immune system efficiency in our biological model waned with age, signifying a deteriorating immune function. It is crucial to note, however, that our inquiry was confined to a single lymphoid organ, the bursa of Fabricius. Consequently, the assertion of a compromised immune status remains tentative, necessitating further investigation across additional immune system components.

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