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A Comparative Study Of Physiological, Hematological, Immune, And Cytomorphometrical Indices In Three Avian Species During The Spring Season In India

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Abstract

The physiological parameters along with cell morphology and morphometry are useful for determining the health or general condition of birds and its environment. So, this study was conducted to determine the hematological and cytomorphometric parameters of three avian species; spotted munia, baya weaver, and redheaded bunting (N = 9 adult male birds/ species). For the hematology and erythrocyte cytomorphometry blood sample was drawn from each bird at sunrise time. We found that, the number of heterophils and body weight significantly differed among the three bird species. MCHC (Mean corpuscular hemoglobin Concentration) and H/L ratio were higher whereas lymphocyte count was lower in redheaded bunting compared to the other two species. Eosinophil count and erythrocyte's N/C area ratio (erythrocyte nucleus area and erythrocyte cytoplasm area ratio) were higher in baya weaver relative to redheaded bunting. The erythrocyte's nuclear area, body temperature, and hemoglobin % were significantly different in baya weaver than the other two species. Spotted munia exhibited a higher blood glucose concentration than redheaded bunting and baya weaver. No significant differences were observed in TLC (Total Leucocyte count), TEC (Total erythrocyte count), MCV (Mean corpuscular volume), MCH (Mean corpuscular hemoglobin), erythrocyte elongation ratio, erythrocyte cell area, basophils, monocytes, hematocrit %, and testicular volume. These results indicate the physiology, immunity, and health status along with baseline values about hematological, physiological, and cytomorphometrical parameters of three species; during the spring season when these birds were in the pre-breeding or non-breeding phase.

Keywords: Cytomorphometry, hematology, H/L ratio, immunity, songbirds, spring season.

Introduction

Hematological and biochemical parameters along with cellular morphology and morphometry are useful for investigation of the health or general condition of birds and its environment (Cooper et al. 1986; Perelman, B 1999). It provides valuable physiological and health information (Harr, 2002; Hauptmanova et al., 2006). Most avian hematology have been undertaken in domestic birds, mainly fowl (Hodges 1979, Dieterlen- Lievre 1988), although some wild birds have also been studied (for review see Sturkie 2021). Besides this, cytomorphometric studies to investigate the shape and size of avian blood cells have been done in different ages of Japanese quail, *Coturnix coturnix japonica* (Puspamitra, S., Mohanty, P.K. & Mallik, 2018), Rhea, *Rhea americana* (Gallo et. al., 2015), guinea fowl (Gupta et al, 2012) and in Muscovy duck (Sulaiman et al, 2010). Additionally, comparative cytomorphological studies on erythrocytes and thrombocytes have been explored of domestic fowl, duck, and quail (Shalini et al. 2018). However, it has been shown that migratory species show seasonal changes in several hematology parameters and such variation also arises in captive animals (Chilgren and DeGraw, 1977; DeGraw et al. 1979).

We know that several factors, including illnesses, nutritional state, bodily condition, sex, age, diet, circadian rhythms, and confinement, can affect hematology and cytomorphometry (Fudge, 2000). The metrics used in hematological investigations in birds include hemoglobin concentration, hematocrit levels, white blood cell (WBC) and red blood cell (RBC) counts, as well as a variety of biochemical indicators. These parameters, which represent adaptations to particular ecological environments and life history aspects, might differ greatly between species. According to Leonard (1982) and Cooper et al. (1986), these indicators are clinically significant for the diagnosis and tracking of avian health issues. For all captive-breeding operations and, whenever feasible, for the populations of sensitive species, a veterinarian diagnostic "screening" using hematological and clinical chemicals is advised (Cooper et al. 1986).

The purpose of the present study is to give insights into the baseline values of selected blood hematological and biochemical parameters in healthy songbirds; including temperate and sub-tropical species, during spring season. These three bird species belong to order- Passeriformes. These bird species are Spotted munia (*Lonchura punctulata*), commonly known as Telia munia or Sinewaz, a resident species belonging to the Estrildidae family. They live in flocks around open cultivation and are distributed all over India except arid portions of Rajasthan and Punjab. Their breeding season is from August to October (Ali and Ripley, 1999). Spotted munia breeds during late autumn with gonads beginning to grow in August/ September, mature in October/ November and post-reproductively regress before the winter solstice. Indian weaver bird (*Ploceus philippinus*), commonly known as baya weaver, is a sparrow-size finch, about 15 cm in length, with a short square-cut tail and stout conical bill, belongs to the family- Ploceidae. These are widely distributed resident species throughout India, Bangladesh, Pakistan, Sri Lanka and Myanmar. Baya weaver breeds during May to September. Redheaded bunting (*Emberiza bruniceps*), commonly known as lal sar ka gandam or surkha, belongs to the family- Emberizidae. They are sexually dimorphic, sparrow-size finch about 17-18 cm in length, have conical bill and noticeable forked tail. Redheaded buntings show sexual dimorphism; males have bright yellow underparts, and a brownish-red face and breast while females are washed-out/duller version of the males. Redheaded buntings are long-distance Palaearctic-Indian migrants. Their breeding grounds are located (~40° N) in south-eastern Europe, Asia minor, Palestine, Syria, Upper Mesopotamia and Persia while their post-reproductive wintering grounds in India (25° N) spreading throughout western and central India. They breed during summer months. It has been observed that buntings spent about 96 days (48 days each way) to cover a migratory journey of about 7,000 km to and fro, about 90 days at breeding grounds; and the remaining days of the year at their overwintering grounds (Ali and Ripley, 1999).

These species respond differently to photoperiod and have different paces of life (Malik et al., 2014), so, we aimed to compare hematology and cytomorphometry in the aforementioned avian species, highlighting the variations in hematological parameters across different species and their ecological and physiological implications. We were curious whether these species show concomitant changes in hematology, cytomorphometry, and physiology or there are existing differences during the spring season.

2.0. Material and methods

2.1. Animals and maintenance

The study was performed on adult males of three avian species; spotted munia (*Lonchura punctulata*) baya weaverbird (*Ploceus philippinus*), and redheaded bunting (*Emberiza bruniceps* after approval from the Institutional Animal Ethics Committee of the University of Lucknow, India (protocol #: LU/ZOOL/IAEC/05/19/11). For this study, we selected 27 adult male birds; 9 birds per species, that were procured from the wild in February. The procured birds were brought to the laboratory and kept in an outdoor aviary (size=3.0×2.5×2.5 m) for a week under natural light and temperature conditions. At this time, the natural photoperiod was about 11.33 h (sunrise to sunset) and daytime temperature varied from 21 to 25 °C. Food and water were provided *ad-libitum*.

2.2. Study

The study was done on spotted munia which is a non-photoperiodic species and its internal clock follows a circannual rhythm, baya weaver which is a resident bird and slow responder to change in photoperiod and other environmental conditions, whereas the redheaded bunting is a migratory bird and shows quick response to photoperiodic changes. After acclimation to captive conditions in the outdoor aviary, in March (Mid-march; during the spring season; day length ~12 hour) different physiological and hematological parameters were measured/ recorded from each individual. Study procedures are detailed henceforth.

2.2a Blood Sampling and cytomorphometry of erythrocytes

Blood was collected by venipuncture of the left-wing vein (*vena ulnaris cutanea*) with 26 number gauge needle. A drop of blood was transferred onto a double-frosted microscope slide (Borosil, 9100D01), and thin blood smears were prepared. Blood smears were air dried, fixed in methanol for 5 minutes, and then stained with Leishman's stain (Bhattacherjee et al., 2017; Puspamitra et al., 2018). For the cytomorphometrical study, we measured the length and width of 50 randomly chosen erythrocytes and their nuclei from each individual, and also derived their area by using the formula $4/3\pi a^2 b$ (a = half of the length of small axis of the cell, b = half of the length of large axis of the cell). Only normal and non-overlapped erythrocytes were measured using Leica application suite (LAS) software v4.12 under 1000x magnification with an oil immersion light microscope equipped with a camera and computer (Leica microsystems DM 3000 and DFC 450c, Germany). Further, we calculated the N/C area ratio (Acharya and Mohanty 2019) and the erythrocyte elongation ratio by dividing erythrocyte length by its width (Soulsbury et al. 2022).

2.2b Leukocyte profile and H/L ratio

For the evaluation of differential leukocyte count (DLC), the blood smears were observed under 1000x magnification using oil immersion microscope and recorded the relative abundance of particular leukocytes (Haile and Chanie, 2014). The first 100 leukocytes per slide were counted and identified as heterophils, basophils, eosinophils, lymphocytes, and monocytes (Campbell, 1995; Matson et al., 2012; Hegemann et al., 2012;). To avoid oversampling and counting differences, the smears were observed by a single person at a time. Further, the heterophil: lymphocyte (H/L) ratio was calculated from the obtained DLC counts as the number of heterophils divided by the number of lymphocytes for each smear (Horak et al., 1998, Vleck et al., 2000).

2.2c. Estimation of total erythrocytes count, and hematocrit

Total Erythrocyte Count (TEC) and Total Leucocyte Count (TLC) were evaluated by a hemocytometer; a Neubauer improved chamber (HBG, Germany) (Campbell, 1995). For hematocrit estimation, the blood was collected into heparinized hematocrit capillary tube. The tube was then sealed and centrifuged for 10 minutes at 10,000 rpm (Schultz et al., 2017, Pap et al. 2010). The column length of the packed cell volume was measured and divided by the length of the whole column of blood (cells and plasma) (Hõrak et al., 1998). To obtain hematocrit, the acquired value was multiplied by 100%.

Further, the other related parameters such as Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin concentration (MCHC) were calculated using standard formulae (Campbell and Ellis 2007; Clark et al., 2009).

2.2d. Physiological indices: body mass, surface body temperature, blood glucose level, hemoglobin and testicular volume

The surface body temperature was recorded from the shank area of the birds just before the blood sampling by quick shot IR ThermoScan, India (Model: EXP-01B) (Kumar et al., 2021). Soon after that, the body mass was measured using a top pan balance up to an accuracy of 0.1 g. For this, the birds were kept inside a cotton bag and weighed quickly. During blood sampling, a drop of blood was placed on a glucose strip attached to a glucometer (Accu-Chek active, Roche Diabetes Care, GmbH) to estimate the blood glucose level (Singh et al., 2016). The concentration of hemoglobin (Hb) was estimated by Sahli's hemoglobinometer following Sahli's method (Sonia et al., 2012). The testicular volume of the left testis was recorded following the unilateral laparotomy protocol as done in previous studies from our lab (Kumar et al.,2001, Kumar et al., 2002).

3. Statistical analysis

After checking the parameters' normality distribution, we performed the ordinary one-way analysis of variance (ANOVA) without repeated measures followed by the Tukey multiple comparison *post-hoc* test on the datasets that passed the test of normality (body mass, surface body temperature, blood glucose, hemoglobin, heterophils, lymphocytes, MCHC, TEC, HCT, H/L ratio, erythrocytes elongation ratio, cell area, nucleus area), and Kruskal Wallis test followed by Dunn's Multiple comparison *post-hoc* test on the datasets that did not pass the normality test (eosinophils, basophils, monocytes, MCV, MCH, TLC, and N/C ratio). Significance was considered at $p < 0.05$. Data is shown in the graphs as mean ± SEM along with the individual's scatter dot plot. All data analyses and graphical designing were done using GraphPad Prism software version 8.0 (San Diego, CA, United States).

4.0. Results:

The results related to leucocyte quantification, hematological, cytomorphometrical, and physiological parameters are shown in Fig. 1-4.

4.1. Effect on physiological parameters

The body weight, body temperature, blood glucose, and hemoglobin of the three species was found to be significantly different from each other ($p < 0.05$; ordinary one-way ANOVA; Table 1). The body weight of spotted munia was minimum followed by baya weaver, while that of redheaded bunting was maximum (Fig.1(i)). Also, the blood glucose level was higher in spotted munia (Fig.1(iii)), body temperature and hemoglobin were lower in baya weaver related to the other two species (Tukey multiple comparison *post-hoc* test) (Fig.1(ii) and Fig.1(iv) respectively). No significant differences in the testicular volume of the species under consideration was found ($p > 0.05$, Kruskal-Wallis test; Fig.1(v); Table 1).

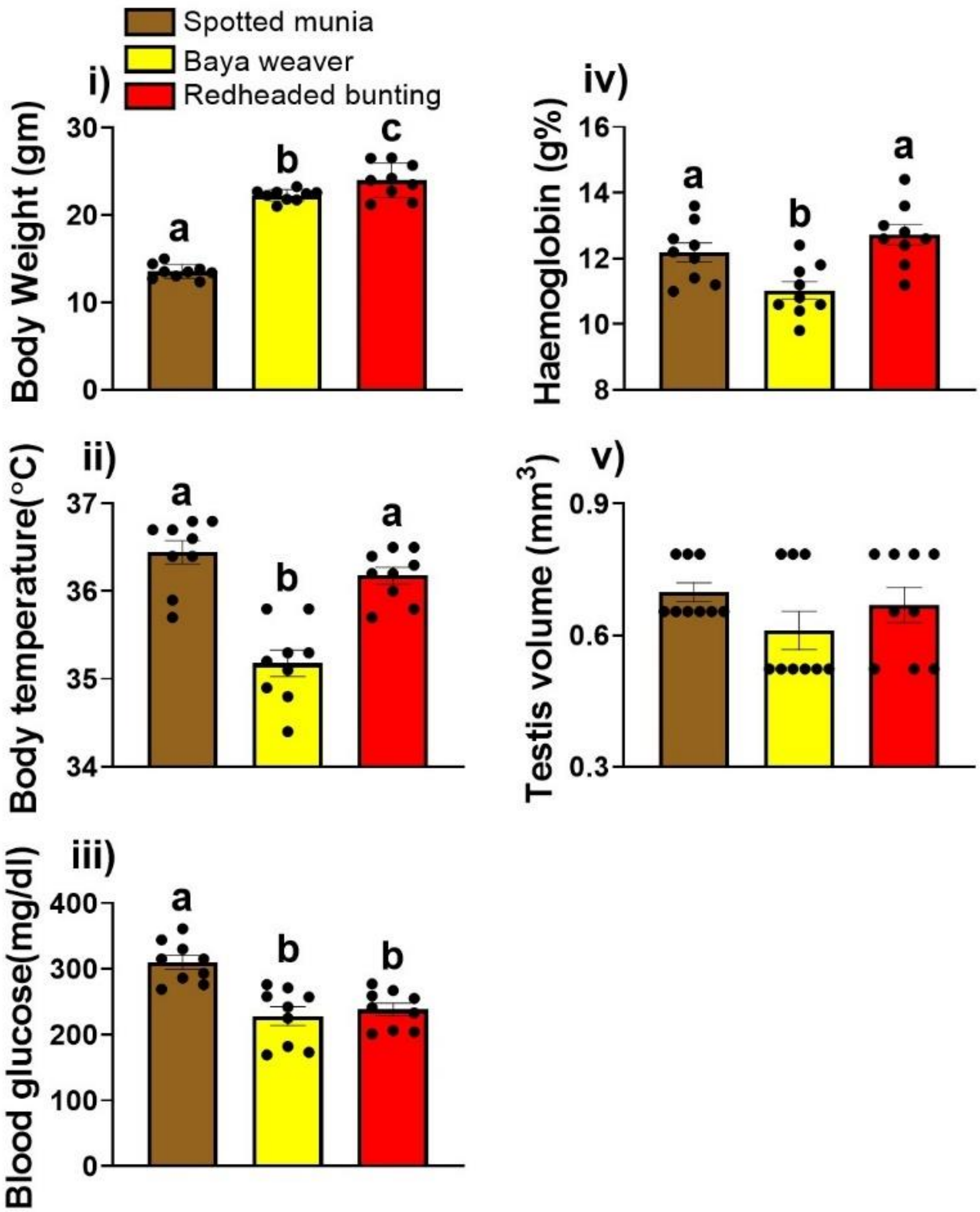

Fig.1 Physiological parameters of the spotted munia, baya weaver, and the redheaded bunting under natural day length. Graphs showing Mean (± SEM) and scattered plot of (a) body weight, (b) body temperature, (c) blood glucose concentration, (d) hemoglobin concentration, and (e) testicular volume of these species. Dissimilar alphabets over the bars indicate points of significant difference among the three species. The alpha was set at 0.05 for statistical significance.

4.2. Effect on hematological parameters

4.2a. Leukocyte quantification

Quantitative analysis of leucocytes showed significant differences in the number of cell-types among the three species (heterophils and lymphocytes: $p < 0.05$, ordinary one-way ANOVA; eosinophils: $p < 0.05$, Kruskal-Wallis test; Table 1) except for the number of monocytes and basophils ($p > 0.05$, Kruskal-Wallis test; Fig.2(iii) and Fig.2(v) respectively; Table 1). The maximum number of heterophils was observed in redheaded bunting followed by spotted munia, and the minimum number was observed in baya weaver (Fig.2(i)) and the number of lymphocytes was minimal in the redheaded bunting than the other two species (Tukey multiple comparison *post-hoc* test; Fig.2(ii)). The eosinophils' number was higher in the baya weaver as compared to redheaded bunting only (Dunn's multiple comparison *post-hoc* test; Fig.2(iv)).

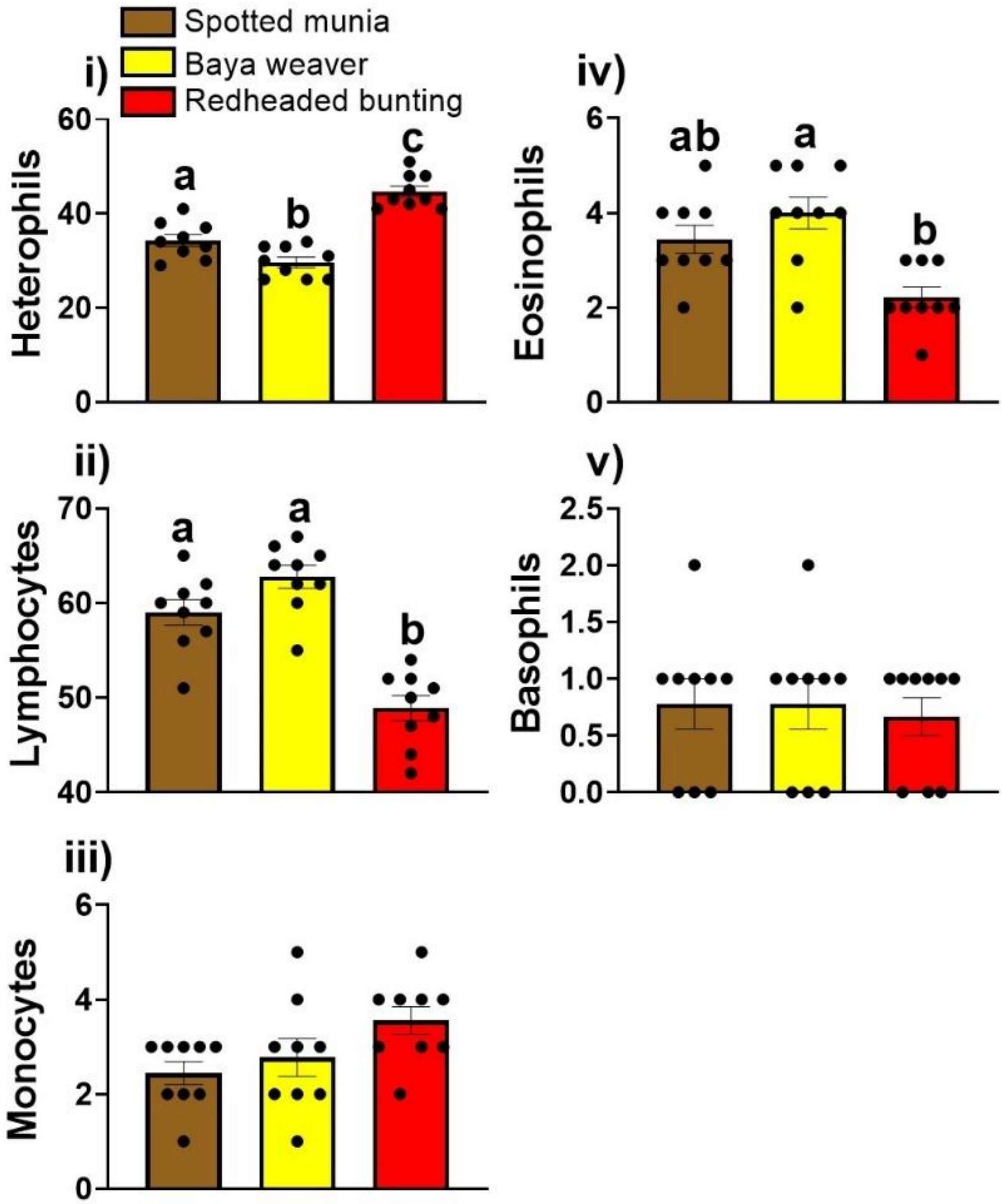

Fig.2 Leucocyte quantification of the spotted munia, baya weaver, and the redheaded bunting under natural day length condition. Graphs showing Mean (± SEM) of (a) heterophils, (b) lymphocytes, (c) monocytes, (d) Eosinophils, (e) basophils number per 100 leucocytes counts of these species. Dissimilar alphabets over the bars indicate points of significant difference among the three species. The alpha was set at 0.05 for statistical significance.

4.2b. Hematological parameters

The MCHC and H/L ratio varied significantly among the three species ($p < 0.05$, Ordinary one-way ANOVA; Table 1). The MCHC and H/L ratio was significantly higher in the redheaded bunting than the other two species (Tukey multiple comparison *post-hoc* test; Fig.3(iii) and Fig.3(vii) respectively). Hematocrit % was statistically significantly different among the three species ($p < 0.05$; Ordinary one-way ANOVA; Fig.3(vi); Table 1). We did not find any significant differences in the TEC ($p > 0.05$; Ordinary one-way ANOVA; Fig.3(i); Table 1), TLC, MHC, and MCV ($p > 0.05$, Kruskal Wallis test; Table 1) among these species. Fig.3(ii),(iv), and (v) respectively.

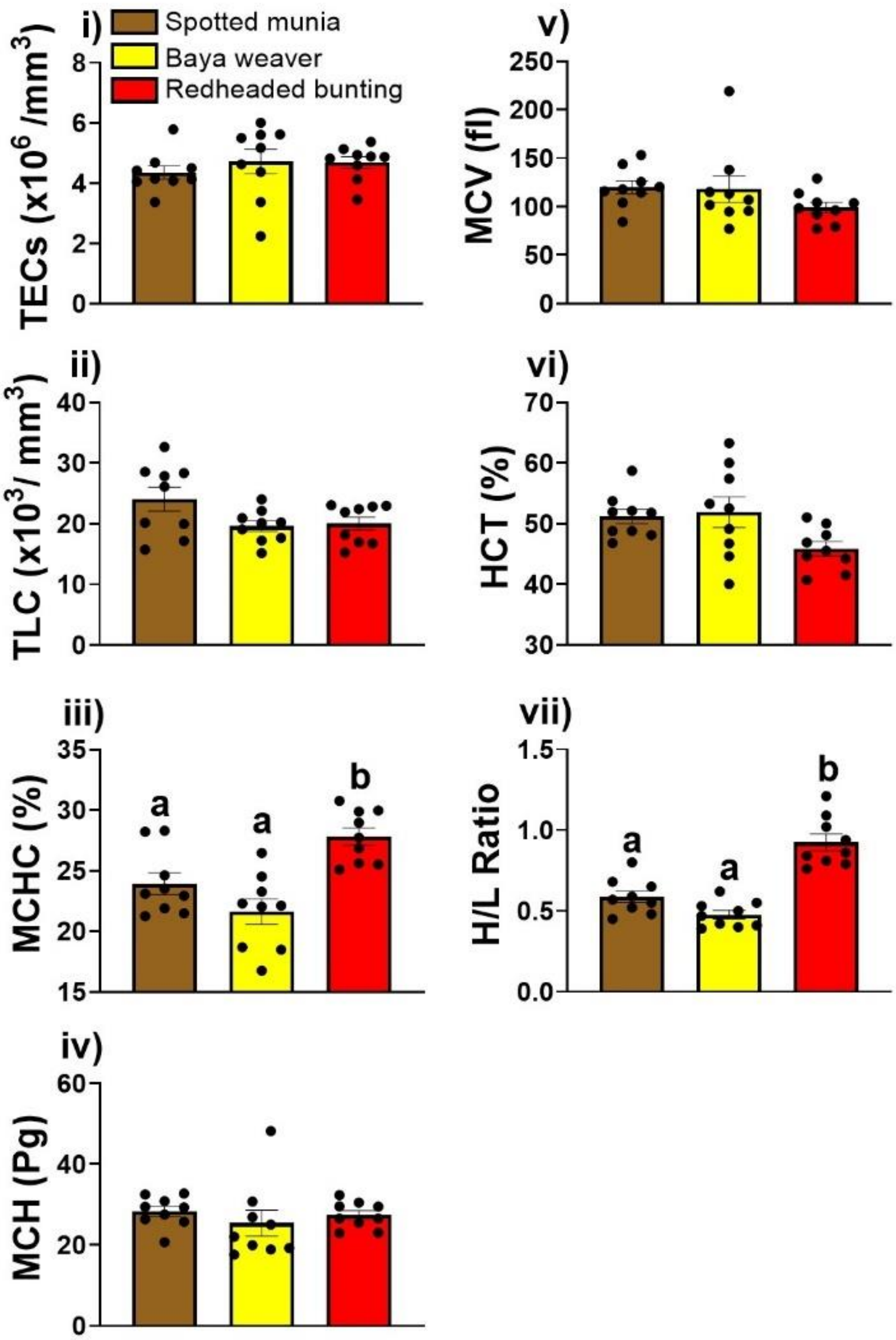

Fig.3 Hematological parameters of the erythrocyte of the spotted munia, baya weaver, and the redheaded bunting under natural daylength. Graphs showing Mean (± SEM) and scattered dot plot of (a) TEC, (b) TLC, (c) MCHC, (d) MCH, (e) MCV, (f) HCT, (g) H/L ratio of these species. Dissimilar alphabets over the bars indicate points of significant difference among the three species. The alpha was set at 0.05 for statistical significance.

4.2.c. Cytomorphometrical analysis

Among the erythrocyte length, erythrocyte width, erythrocyte nucleus length and the erythrocyte nucleus width, only the erythrocyte nucleus length showed the significant difference among the three bird species ($p < 0.05$, Ordinary one-way ANOVA; Fig 4.(i), (ii), (iii), (iv); Table 1). Erythrocyte nuclear length was maximum in the baya weaver compared to the spotted munia and the redheaded bunting (Tukey multiple comparison *post-hoc* test). Cytomorphometric analysis also showed a significant difference in the erythrocyte's nucleus area ($p < 0.05$, Ordinary one-way ANOVA; Fig 4.(vi); Table 1) and the erythrocyte N/C ratio ($p <0.05$; Kruskal Wallis test; Fig 4.(vii); Table 1) among the three species. The nucleus area of the baya weaver was higher than the spotted munia and the redheaded bunting, whereas, the nucleus area of the spotted munia was similar to redheaded bunting (Tukey multiple comparison *post-hoc* test). The erythrocyte N/C ratio of the baya weaver was higher than the redheaded bunting whereas that of spotted munia was similar to the other two species (Dunns multiple comparison *post-hoc* test). However, the differences in erythrocyte cell area and cell elongation ratio were insignificant among the three species ($p > 0.05$; Ordinary one-way ANOVA; Fig 4.(v) and (viii) respectively; Table 1).

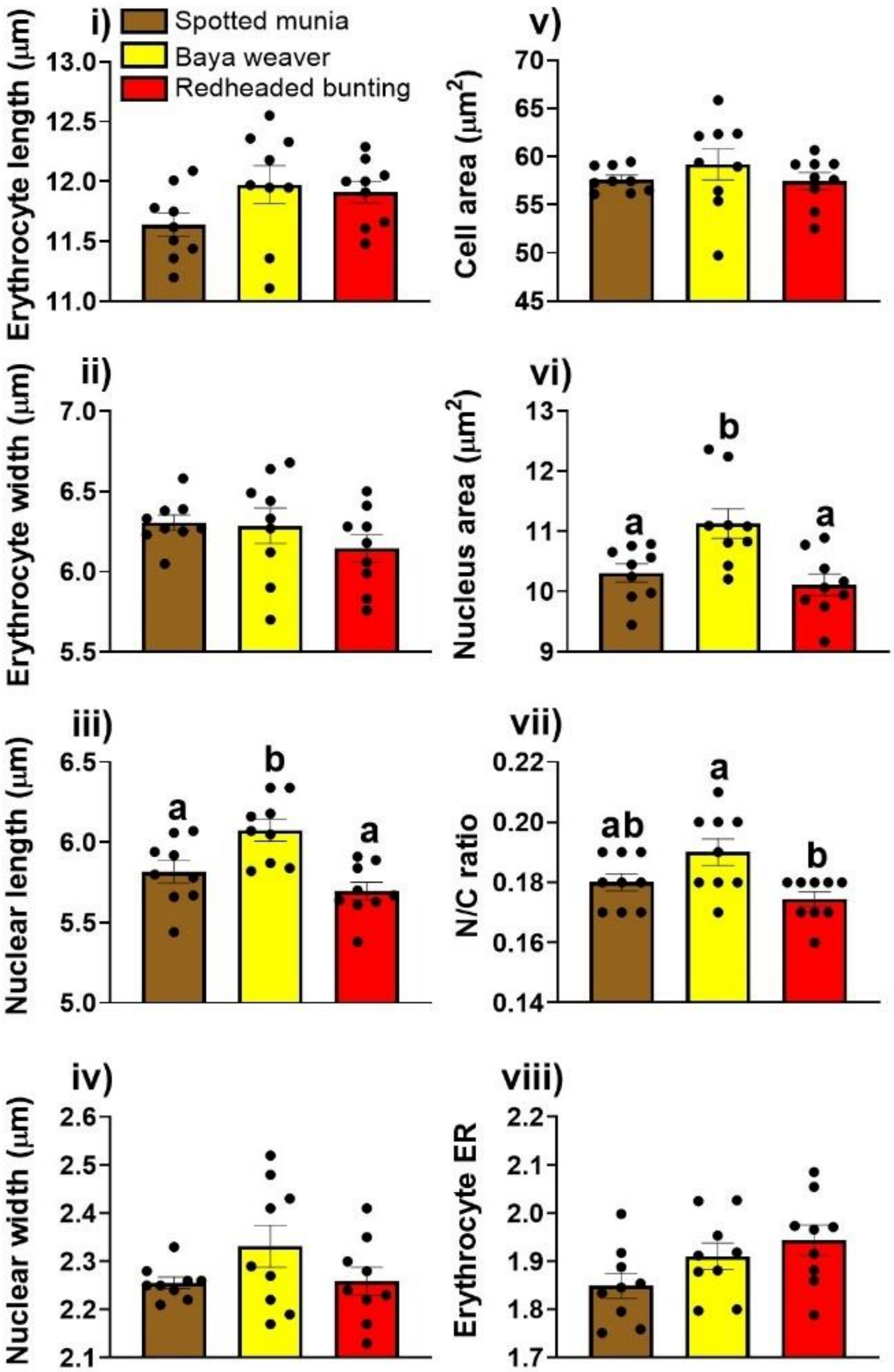

Fig.4 Cytomorphometrical data of the erythrocyte of the spotted munia, baya weaver, and the redheaded bunting under natural daylength. Graphs showing Mean (± SEM) and scattered dot plot of (a) erythrocyte length, (b) erythrocyte width, (c) nucleus length, (d) nucleus width, (e) cell area, (f) nucleus area, (g) N/C ratio, and (h) erythrocyte elongation ratio of these species. Dissimilar alphabets over the bars indicate points of significant difference among the three species. The alpha was set at 0.05 for statistical significance.

Table 1. The table shows the physiological parameters,leucocyte quantification, hematological parameters, and erythrocyte cytomorphometry data of the three observed species; spotted munia, baya weaver, and redheaded bunting along with their *p*-value, F-value or KW value. The bold numerals represent the significant values having p <0.05.

Parameters	*p*-value	$F_{(2, 24)}$ / KW value
Physiological parameters		
Body weight	**<0.0001**	**167**
Body temperature	**<0.0001**	**26.96**
Blood Glucose	**<0.0001**	**14.67**
Hemoglobin	**0.0014**	**8.750**
Testis volume	0.2603	2.692
Leukocyte quantification		
Heterophils	**<0.0001**	**41.32**
Basophils	0.9389	0.1261
Eosinophils	**0.0024**	**12.08**
Lymphocytes	**<0.0001**	**30.90**
Monocytes	0.0530	5.876
Hematological parameters		
TEC	0.6137	0.4984
TLC	0.2509	2.765
MCHC	**0.0002**	**12.33**
MCH	0.1440	3.877
MCV	0.0724	5.252
HCT	**0.0439**	**3.571**
H/L Ratio	**<0.0001**	**35.31**
Cytomorphometry		
ythrocyte Length	0.1322	2.204
Erythrocyte Width	0.3512	1.093
Erythrocyte Nucleus Length	**0.0011**	**9.158**
Erythrocyte Nucleus Width	0.1722	1.895
Cell area	0.4760	0.7658
Nucleus Area	**0.0027**	**7.659**
N/C Ratio	**0.0307**	**6.967**
Elongation Ratio	0.0774	2.852

5.0. Discussion

Three bird species—spotted munia, baya weaver, and redheaded bunting—were compared in terms of their springtime cytomorphometry, erythrocyte cytomorphology, and physiological reactions. Erythrocyte morphometry and hematology are crucial for identifying both pathological and physiological aspects of birds' health. These investigations can offer blood cell morphometry as well as differential diagnosis for hematological and physiological status. Reliable information on metabolic diseases, nutrition, chronic stress status, and immune aspects can be obtained by clinically analysing the avian health condition by blood cell morphology investigation.

5.1. Physiological parameters

The results showed that the body weight of spotted munia was minimum followed by baya weaver, while that of redheaded bunting was maximum. Since spotted munia is a small bird compared to sparrow-sized baya weaver and redheaded bunting (Ali and Ripley 1999), the differences in body mass becomes obvious. Our finding is similar and within range to the previous studies on these birds (Bhatt and Chandola 1982, Budki et al, 2014). Interestingly, spotted munia and redheaded bunting showed the higher body temperature value since the prior species is smaller in size and the latter one was in the preparatory phase for migration, however, baya weaver showed the lowest body temperature value as it is larger than spotted munia because large size birds tend to have low body temperatures and small birds have high body temperatures (Rodbard, 1950), and also baya weaver had no pre-migratory stress. Besides that, baya weaver is the late responder to the environmental cues, it takes a long span of time to show any change at the physiological level. Further, at the physiological level, we measured blood glucose levels in birds as this parameter is easy to observe and instantly provides information about the energy requirements of the birds. Blood glucose is utilized by birds for a variety of functions mainly being energy production and its concentration is maintained at basal level (Klasing, 1998).

In our study we found that blood glucose concentration was highest in spotted munia compared to the baya weaver and the redheaded bunting possibly because of the differences in their body sizes and thus differential metabolic requirements. However, there was no significant difference in the blood glucose level in the baya weaver and redheaded bunting, since the body size of redheaded bunting is larger than the baya weaver bird, and this was the preparatory phase of the redheaded bunting for spring migration, during which the metabolic activities become higher than the non-migratory phase. As metabolism is associated with the blood glucose levels, although in a non-linear way, blood glucose often tend to be high in small, altricial species (Beuchat and Chong 1998; Braun and Sweazea 2008).

5.2. Hematological parameter

5.2a. Leukocyte quantification

In avian species, heterophils and lymphocytes make up about 80% of the total leukocyte population (Rupley 1997). Lymphocytes are essential for various immunological functions, such as producing immunoglobulins and regulating immune responses (Campbell 1996). Heterophils act as the main phagocytic leukocytes and their numbers increase in the bloodstream in response to infections, inflammation, and stress (Campbell 1995; Rupley 1997; Thrall 2004). The remaining 20% of leukocytes include eosinophils, which are involved in the inflammatory response and play a role in defending against parasitic infections (Rupley 1997; Kiesecker 2002), monocytes, which are long-lived phagocytic cells that help combat infections and bacteria (Campbell 1995; Davis, Cook & Altizer 2004), and basophils, whose exact function is not well understood (Rupley 1997) but is thought to be associated with inflammation (Campbell 1995). The quantitative analysis of leukocytes showed significant differences in the number of cell-types among the three species. The highest number of heterophils was observed in redheaded bunting followed by spotted munia, and the lowest number was observed in baya weaver. It might be possible as spring season is considered pre-breeding or non-breeding season for these birds so these have a stronger immunity, as there is no trade-off with the reproductive system. However, the number of lymphocytes was lower in the redheaded bunting than in the other two species which might indicate that pre-breeding preparation for a migratory bird requires a low lymphocyte count compared to non-migratory resident bird species spotted munia and baya weaver. Higher eosinophils' number in the baya weaver as compared to redheaded bunting may be due to slight inflammation in body during this time of the year, which may need further investigation in future research. It is anticipated that the immune system will modify its defense mechanism according to the season, as immune system activation is an energy demanding process and the increased exposure to parasites is predictable (Hasselquist 2007) as an adaptive response involving, and increased number of heterophils in the blood stream (Pap et al. 2010). When there is no energetic incompatibility between the immune system and breeding season, there is an increase in the number of lymphocytes in the blood stream (Hasselquist 2007) and consequently a lower H/L ratio.

5.2b. Hematological parameters

A higher H/L ratio observed in the redheaded bunting than the other two species might indicate pre-breeding stress owing to the preparatory phase for migration. The H/L ratio is considered a reliable stress index, frequently used in ecological studies of birds (Moreno et al. 2002, Ots et al., 1998). Hemoglobin was significantly lower in the baya weaver than in the other two species might be due to lower oxygen demands in that particular season and life history state. The blood hemoglobin concentration, also indicates the health status of the birds (O'Brien *et al.*, 2001). Contrary to this, in other non-migratory passerine species, no changes have been reported in hemoglobin levels between seasons (Breuer *et al.*, 1995). Since hemoglobin is the most crucial hematological indicator to indicate oxygen-carrying capacity and directly reflects oxygen demand (Minias, 2015), it is probable that an increase in energic changes in Hb and HCT at the start of breeding in Thrushes (Provinciato et al., 2024). The higher MCHC in the redheaded bunting might be due to increased hemoglobin levels. These species had no significant differences in the TEC, HCT, TLC, MHC, and MCV parameters. These indices were similar among the three species studied and compared to those levels reported for other avian species (Hawkey et al. 1991; Campbell 1995). The physiological condition of birds has also been commonly assessed using hematocrit (packed cell volume), which is the percentage of blood volume occupied by erythrocytes (Harrison and Harrison, 1986). Although hematocrit and hemoglobin concentrations are expected to be correlated in birds (Velguth *et al.*, 2010) but in our study no significant differences in hematocrit values among the three species might be because they were almost in a similar life history state.

5.2c. Cytomorphometrical analysis

Cytomorphometric analysis of erythrocytes were uniform in the three species analyzed except for differences in the erythrocyte's nucleus area, N/C ratio, and nuclear length. The nucleus area and length of the baya weaver were higher than the spotted munia and the redheaded bunting whereas, nucleus area of spotted munia was identical to redheaded bunting. It has been noted that a smaller and more condensed nucleus permits the existence of a proportionally larger cytosome and hence a greater content of Hb (Lucas & Jamroz 1961). So, this might be the reason for low hemoglobin, similar MCHC, and TEC in baya weaver. The erythrocyte N/C ratio of the baya weaver was higher than the redheaded bunting whereas that of spotted munia was similar to the other two species. The altered nuclear shape may be possible due to changes in the nuclear lamina (Webster et al. 2009). The sizes of erythrocytes can show plasticity in response to surrounding temperature (Ruiz et al. 2004). However, differences in erythrocyte cell area and cell elongation ratio were similar among the three species. It may suggest ellipsoidal shape of erythrocytes to be most effective in gaseous exchange (Janiga et al. 2017; Bhattacherjee 2023).

6.0. Conclusion

This study presents information on comparative hematology and cytomorphometry of three birds; baya weaver (*Ploceus philippinus*), spotted munia (*Lonchura punctulata*), and red-headed bunting (*Emberiza bruniceps*) which can serve as a means to evaluate song birds' physiological conditions, immunity and health status, particularly in the spring season. Hematology and erythrocyte morphometry play an important role in diagnosing physiological and pathological aspects of birds' health. The present data represent a baseline value, which may provide a better understanding of different songbirds during the spring season, as the threshold values of hematological data, for such parameters in avian medicine remain incomplete and inadequate. Such studies may provide differential diagnoses of hematological and physiological conditions along with morphometry of blood cells. By investigating the morphology of blood cells, the avian health status can be clinically analyzed to understand reliable information on metabolic disorders, nutrient deficiencies, chronic stress, and pathological aspects can be provided.

Author statement: AK: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization and Writing - original draft; **VV:** Data curation; Investigation; Methodology; Validation; Visualization and Writing- review & editing; **SM:** Project administration; Resources; review **SR:** Conceptualization; Funding acquisition; Resources; Software; Supervision; Validation; Visualization and Writing - review & editing.

Declaration of competing interest: The authors declare that they have no competing interests.

Data availability: Datasets will be made available upon reasonable request from the corresponding author SR.

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