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Comparative blood cell morphometry and differential leukocyte count of two breeds of turkey, *Meleagris gallopavo* (Linnaeus, 1758)

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Morphometry of erythrocytes and leukocytes and differential leukocyte count of two breeds of adult turkey (24 birds) were performed with respect to sexual dimorphism. Except nuclear length of erythrocytes, other parameters show highly significant difference at $P < 0.01$. Leukocytes reflected significant difference at $P < 0.01$ among and between breeds with respect to their dimensions. In case of DLC, except eosinophils, all leukocytes show significant difference ($P < 0.01$) among and between breeds. Morphometry of blood cells of two breeds of turkey is within the range mentioned for avian species, but the differential count revealed some abnormalities which might be due to stress or infection.

Keywords: Blood cell, differential leukocyte count, turkey, *Meleagris gallopavo*, morphometry.

CYTOMORPHOMETRY of blood cells, an important aspect of hematology, can reveal the physiological condition of organisms. In some birds, cytomorphometry of erythrocytes has only been reported[1–5]. The morphology and morphometry of both erythrocytes and leukocytes were earlier discussed in adult male ostrich[6]. But studies on nuclear morphometry of blood cells are inadequate in birds[7]. Measurements of both cellular and nuclear length and breadth of erythrocytes, lymphocytes and monocytes, cellular diameter of granulocytes and cellular dimensions of thrombocytes were earlier reported in different chickens[8]. But comparison of these parameters between those birds, especially with respect to breed and sex, is not reflected in their studies. In case of turkeys, data on blood cell morphometry are scanty. Many birds do not express clinical signs until late stages of the disease and the signs that they do exhibit may be subtle and non-specific[9] where DLC can be used as a valuable tool to determine the health status, genetic disease resistance, meat quality, stresses due to environment, nutritional, and pathological factors. The present study is an attempt to report breed-wise differences in morphometry of blood cells and DLC of turkey.

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Table 1. Morphometry of blood cells of two breeds of turkey

Type of cell	Cell/nucleus	Parameters	White breed		Bronze breed		*F*-value
			Male (30)	Female (30)	Male (30)	Female (30)	
Erythrocyte	Cell	Length	13.26 ± 0.17^{a}	14.10 ± 0.33	14.41 ± 0.15^{a}	14.75 ± 0.30^{a}	6.40**
		Breadth	8.19 ± 0.14	7.91 ± 0.19^{b}	9.17 ± 0.18^{b}	8.74 ± 0.18	3.70**
	Nucleus	Length	6.91 ± 0.21	6.19 ± 0.25	6.27 ± 0.24	6.33 ± 0.26	1.83^{NS}
		Breadth	4.36 ± 0.11^{a}	$3.32 \pm 0.12^{a,b}$	4.11 ± 0.16^{b}	3.64 ± 0.15^{a}	10.63**
			Male (30)	Female (30)	Male (30)	Female (29)	
Lymphocyte	Cell	Length	9.94 ± 0.41^{a}	$7.56 \pm 0.24^{a,b}$	$11.32 \pm 0.37^{a,b}$	11.07 ± 0.40^{b}	21.26**
		Breadth	8.83 ± 0.42^{a}	$6.94 \pm 0.19^{a,b}$	$10.67 \pm 0.29^{a,b}$	9.63 ± 0.35^{b}	23.18**
	Nucleus	Length	6.92 ± 0.29^{a}	$5.27 \pm 0.19^{a,b}$	$8.19 \pm 0.27^{a,b}$	$8.35 \pm 0.44^{a,b}$	21.18**
		Breadth	6.35 ± 0.30^{a}	$5.32 \pm 0.19^{a,b}$	$7.76 \pm 0.23^{a,b}$	$7.75 \pm 0.34^{a,b}$	18.56**
			Male (30)	Female (30)	Male (30)	Female (30)	
Monocyte	Cell	Length	10.88 ± 0.40^{a}	$11.19 \pm 0.32^{a,b}$	$12.36 \pm 0.39^{a,b}$	$11.90 \pm 0.37^{a,b}$	18.56**
		Breadth	9.38 ± 0.41^{a}	10.14 ± 0.29^{b}	$11.82 \pm 0.30^{a,b}$	11.09 ± 0.32^{a}	10.17**
			Male (30)	Female (30)	Male (30)	Female (30)	
Eosinophil	Cell	Length	10.85 ± 0.46^{a}	10.61 ± 0.44^{b}	$13.17 \pm 0.46^{a,b}$	11.68 ± 0.45	6.32**
		Breadth	10.60 ± 0.33^{a}	9.31 ± 0.28^{b}	11.83 ± 0.35^{b}	$11.93 \pm 0.44^{a,b}$	11.68**
			Male (30)	Female (30)	Male (30)	Female (30)	
Heterophil	Cell	Length	9.21 ± 0.32^{a}	$10.78 \pm 0.39^{a,b}$	$13.62 \pm 0.41^{a,b,c}$	$11.52 \pm 0.29^{a,c}$	25.55**
		Breadth	9.18 ± 0.31^{a}	10.14 ± 0.45^{b}	$12.59 \pm 0.29^{a,b,c}$	9.94 ± 0.34^{c}	17.1**
			Male (30)	Female (30)	Male (30)	Female (17)	
Basophil	Cell	Length	10.25 ± 0.33^{a}	9.54 ± 0.30^{b}	11.11 ± 0.30^{b}	$11.82 \pm 0.28^{a,b}$	8.61**
		Breadth	9.72 ± 0.26^{a}	9.24 ± 0.38^{b}	10.82 ± 0.23^{b}	$10.91 \pm 0.33^{a,b}$	6.77**

Mean ± SE with similar superscripts (a, b, c) in the same row differ significantly at $P < 0.01$. Significant at $^{**}P < 0.01$, NS, Not significant. Figures in parentheses represent number of cells observed in each case.

The study was conducted on two breeds of turkey (*Meleagris gallopavo*) being maintained at the Central Poultry Development Organization (CPDO), Eastern Region (ER), Government of India, Bhubaneswar (Odisha), under standard farm management practices. Blood samples were collected from 24 adult birds of which 12 (6 males and 6 females) were of broad-breasted white (BBW) breed and another 12 (6 males and 6 females) broad-breasted bronze (BBB) breed.

Samples were taken out aseptically with the help of sterile 25 gauge needles (Dispo Van single use needle, Hindustan Syringes & Medical Devices, Faridabad) from the wing vein known as ulnar vein of the birds[10]. Blood smears were prepared at site on clean grease-free slides (Blue Star Pic-2, Polar Industrial Corporation, Mumbai), air dried and stained with Leishman's stain (Qualigens Product No. 38854, Leishman's Stain, Thermo Fisher Scientific, Mumbai) for cytomorphometrical analysis on subsequent days. Photomicrographs and cytomorphometry of blood cells of both the breeds with sexual dimorphism were undertaken using a microscope eyepiece digital camera (CatCam130 – 1.3 Mega Pixel (MP), Code No. CC130, Catalyst Biotech, Maharashtra) attached to Hund Wetzlar Microscope (MICROSCOPE H 600 WILOZYT PLAN, Serial No. 1024980, Helmut Hund GmbH, Wetzlar-Nauborn, Germany) and computer. To evaluate DLC, the method given by Nowaczewski and Kontecka[5] was followed.

The entire data from males and females of both breeds were subjected to palaeontological statistics (PAST) (version 2.17, Natural History Museum, University of Oslo) for one-way analysis of variance (ANOVA) followed by Tukey's pair-wise comparison tests. Differences were classified as significant at $P < 0.01$.

The cellular and nuclear dimensions (length and breadth) of RBC and one of the five WBCs, i.e. lymphocyte (one agranulocyte) were measured (Table 1) as they have symmetrical cellular and nuclear boundaries (Figure 1). For other leukocytes (one agranulocyte and three granulocytes), only dimensions of cellular measurement were noted (Figure 2) because they have either indented (monocyte nucleus) or lobed (eosinophil, heterophil and basophils' nuclei) nuclei. Due to this reason, measurement of the length and breadth of their nuclei were not possible. Except the nuclear length of erythrocytes, all other parameters show significant difference among the row at $P < 0.01$. The breadth of RBC differs ($P < 0.01$) between female BBW breed and male BBB breed. All other cells, i.e. leukocytes reflected significant difference ($P < 0.01$) among and between breeds with respect to their parameters. The lymphocytic parameters exhibit significant differences among BBW breed and between the two breeds. The monocyte and eosinophil breadths do not differ among breeds but differ between breeds. Moreover, eosinophil length of BBB hen does not differ with other such groups. Out of all blood cells, only dimensions

of heterophils are significantly different among hens and toms of BBB breed. However, the breadth of heterophils does not differ among males and females of BBW. In case of morphometry of basophils, no significant differences were observed within the breeds though differences exist between toms of BBW breed and hens of BBB and vice versa.

The differential leukocyte count (in %) is reported in Table 2. Except eosinophils, all other leukocytes show significant difference ($P < 0.01$) between the breeds. Female BBW does not show difference with other such groups with respect to percentage of lymphocytes. Monocytes differ significantly among the rows, but interbreed differences were absent. Both intra- and inter-breed differences exist in the case of heterophils. The males of BBW and females of BBB differ significantly (Figure 3). The shapes of blood cells (Figure 4) of these breeds of turkey were quite different from other birds in general. Instead of horse shoe-shaped nucleus, monocytes show variable structures with respect to their shape of nucleus although typical monocytes were also present. Similar is the case for heterophils where instead of multi-lobed nucleus, nucleus was present just near the plasma membrane and without any lobe, as if it juxtaposed with the plasma membrane. Lymphocytes and basophils are almost similar to the other avian species belonging to the order Galliformes. Eosinophils are large and have clear granules and lobed nucleus. But the plasma membrane is not visible as if these cells are swollen.

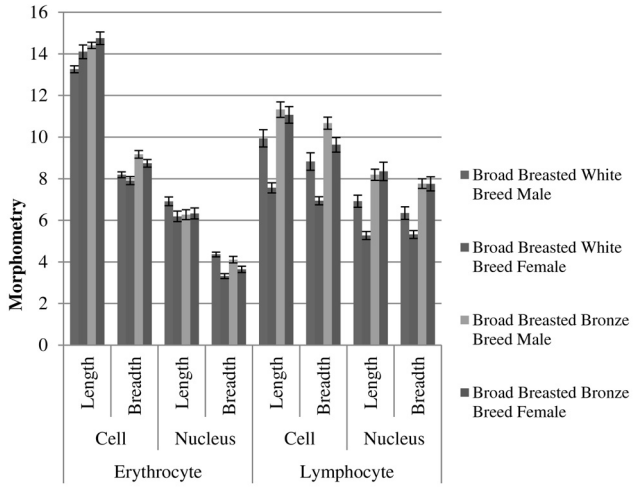

Figure 1. Cellular and nuclear dimensions of erythrocytes and lymphocytes of two breeds of turkey.

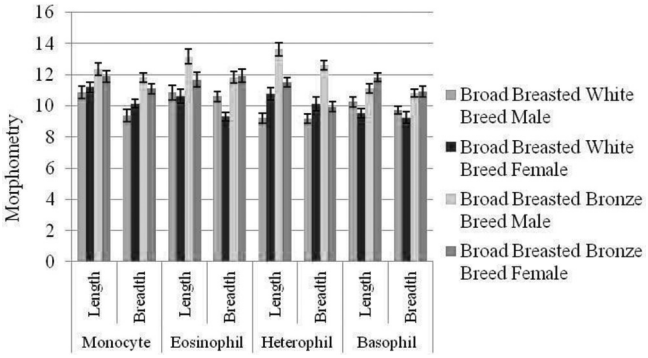

Figure 2. Dimensions of monocytes and granulocytes of two breeds of turkey.

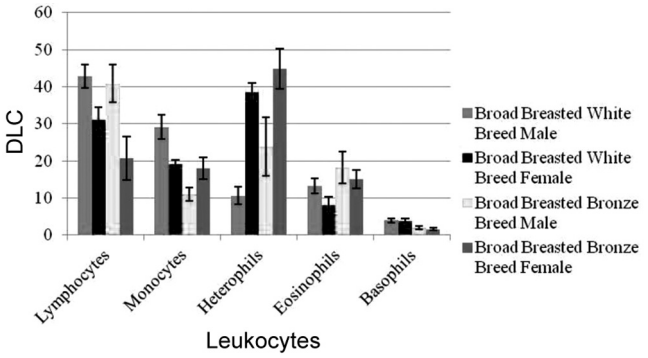

Figure 3. Differential leukocyte count of two breeds of turkey.

The size of avian erythrocyte differs from species to species, but they generally range between 10.7×6.1 μm and 15.8×10.2 μm (ref. 11). The present result agrees with this range. The length of granulocytes of BBW adult individuals in the present study is in accordance with the adult Japanese quail[12]. The dimensions of RBC, monocytes and heterophils of adult common kestrel match with that of both breeds considered for the present study[13]. Also, the dimensions of erythrocytes, heterophils and eosinophils of Sarus crane are in accordance with this study[7]. These similarities might arise due to the large body size. Moreover, dimensions of erythrocytes, basophils and monocytes of male birds investigated in this study are in accordance with adult male ostrich[6] which is also a flightless bird. Heterophils of BBW hens approximately corroborate with that of 30–32 week old male and female bronze turkey[14]. Similarly, heterophils of BBB male and basophils of BBB females match with that of 119 day old B.U.T.6 hybrid male turkey from Romania[15] as this hybrid grows faster and acquires weight like that of other adult turkeys in a short span of time. Lymphocytes of males of both breeds considered for this study are similar to those of Nigerian male and female turkeys[16]. Other than turkeys, the results of this study also corroborate with different poultry birds. Adult helmeted Guinea fowl has similar lymphocyte value as shown by female BBW breed[17]. The presence of lymphocytes and basophils in DLC of BBB female is almost similar to that of adult female and male Bali ducks respectively[18]. Lymphocytes and basophils of adult common crane match with that of female BBW and BBB respectively[19]. Basophils of adult gulls corroborate with that of BBW female[20]. Also, the basophils of adult Canada geese match with that of BBB females[21]. The percentage of lymphocytes in adult Nigerian duck during wet season corroborates with BBW female as these samples were also collected during rainy season[22]. Heterophils of male BBB match with that of captive Puna ibis[23] which may be due

Table 2. Differential leukocyte count of two breeds of turkey

Leukocytes	Broad-breasted white breed		Broad-breasted bronze breed		*F*-Value
	Male (6)	Female (6)	Male (6)	Female (6)	
Lymphocytes	42.83 ± 3.21[a]	31.16 ± 3.23	40.83 ± 5.06[b]	20.66 ± 5.90[a,b]	5.08**
Monocytes	29.16 ± 3.35[a]	19.16 ± 1.07[a]	11.00 ± 1.91[a]	18.00 ± 2.88[a]	9.18**
Heterophils	10.66 ± 2.40[a]	38.50 ± 2.45[a]	23.83 ± 7.84[b]	44.83 ± 5.36[a,b]	9.17**
Eosinophils	13.33 ± 2.06	8.00 ± 2.28	18.16 ± 4.26	15.16 ± 2.48	2.16[NS]
Basophils	4.00 ± 0.57[a]	3.80 ± 0.58	2.00 ± 0.54	1.60 ± 0.40[a]	4.36**

Mean ± SE with similar superscripts (a, b) in the same row differ significantly at $P < 0.01$. Significant at $^{**}P < 0.01$, NS, Not significant. Figures in parentheses represent number of cells observed in each case.

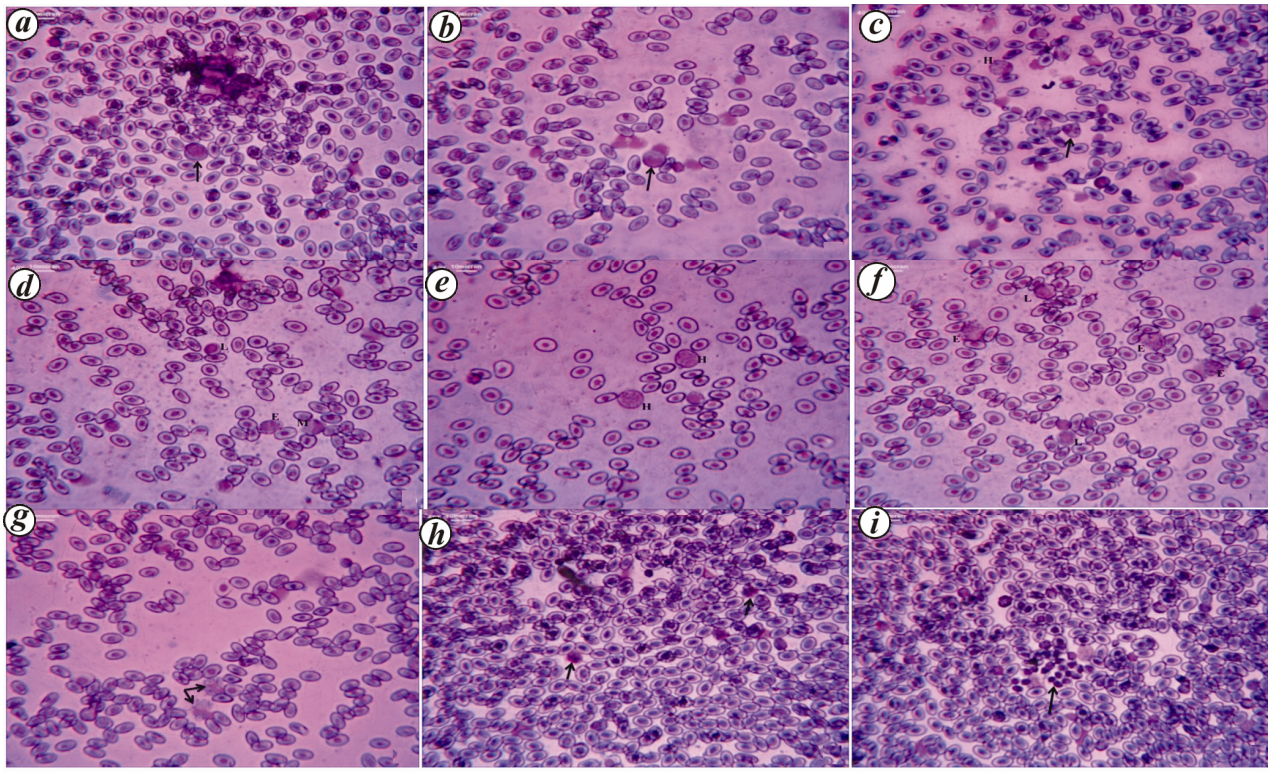

Figure 4. Different blood cells of two breeds of turkey: ***a***, Large Lymphocyte; ***b***, Monocyte; ***c***, Monocyte with a flower-shaped nucleus and heterophil; ***d***, Lymphocyte, eosinophil and monocyte; ***e***, Banded heterophils; ***f***, Lymphocytes and eosinophils; ***g***, Eosinophils; ***h***, Basophils; ***i***, Thrombocytes. (Scale: 40× 10 µm, Figures are captured at 40× with scale length 10 µm which is according to preloaded software of Scopetek device mentioned in text).

to collection of sample at almost the same temperatures. Monocytes and basophils of BBB males and females and heterophils of BBW females match with that of adult budgerigans[24]. Sexually mature domestic pigeon and African collared dove show similar percentage of heterophils with BBW and BBB females respectively. Also collared doves mentioned in the same study have similar amount of basophils compared to BBB female of the present study[25]. Heterophils and lymphocytes of adult common kestrel match with female BBB and males of both breeds respectively[13]. Since these samples were collected from adult birds during day-time, observation regarding the lymphocyte percentage of BBB female is in accordance with that of breeding Great Tits female[26]. Data similar to that of adult female and male captive hill mynah are reflected by BBB females and males of both categories respectively, regarding percentage of heterophils and lymphocyte[27]. Eosinophils of female Galápagos penguins[28] and heterophils of adult male pheasants[4] are like that of female BBW and BBB male respectively. Moreover, the lymphocytes of adult male ostrich are similar to the present study[6]. The increased percentages of eosinophils (eosinophilia) and monocytes (monocytosis) may be due to infections. Heteropenia may have occurred due to decrease in survival rate of mature heterophils or the stored amount of heterophils is exhausted due to fight against inflammation. Also, basophils are more in some cases than the usual range[29]. It may be due to inflammation which is confirmed from the previous study[30].

The deviations of the above mentioned three types of WBCs may be related to infected conditions of these

birds. Similar as well as dissimilar structures of leukocytes from typical ones are supported by previous studies[9,15,31]. Monocytes in the same blood sample may vary in shape. The morphometry of blood cells of two breeds of turkey is within the range mentioned for avian species but the differential count revealed some abnormalities which may be due to stress or infection. Therefore, detailed analyses with respect to these conditions can reflect new aspects.

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