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A Study on Effect of Habitat on Cytomorphometry of Poultry Birds

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ABSTRACT

Cytomorphometry is a quantitative measurement of cellular features. It measures the length and breadth of blood cells using the morphometrical parameters. The present study aims at the evaluation of comparative cytomorphometrical parameters of Vanaraja and Geese being maintained at Centurion University of Technology and Management, Jatani, Bhubaneswar, Odisha. The blood samples were collected from the birds. Smears were prepared and stained with Giemsa stain for morphometrical analysis of blood cells. In case of erythrocyte nuclear length and N/C ratio differ significantly at $p < 0.01$, the cell length, cell breadth and cell area were varies significantly at $p < 0.001$, nuclear area differ significantly at $p < 0.05$ and nuclear breadth reflects non significant value with respect to different habitat of species. The cell length, cell breadth, cell area and nuclear area of lymphocyte significantly differ at $p < 0.001$ but nuclear length, nuclear breadth and N/C ratio don't reflects any significant value. In case of monocyte, cell length, cell area significantly differ at $p < 0.001$ and cell breadth is significant at $p < 0.05$. The cell length, cell breadth and cell area of basophil reflects significantly at $p < 0.001$. The eosinophil of chicken and geese differ nonsignificantly with respect to different habitat. In case of heterophil, cell length differ at $p < 0.01$, cell breadth and cell area are varies at $p < 0.05$. This study shows different size of blood cells in aquatic (Geese) and terrestrial (Vanaraja) due to higher metabolic rate, environmental effect like temperature, oxygen level and some infectious disease.

KEYWORDS: Cytomorphometry, Aquatic, Terrestrial, Geese, Vanaraja, Habitat.

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I. INTRODUCTION

Cytomorphometry is a quantitative process. That measure the cellular features, inputting the data points into a computer via a graphic interface, with the intent of standardizing image analysis. Study of blood cells in many different habitats provides an interesting comparison of cell size in relation to their activities¹. Image analysis is performing to obtain cell diameter, cytoplasmic area, nuclear area, nuclear to cytoplasmic ratio. It also measure length and breadth of erythrocytes, lymphocytes, eosinophils, monocytes, heterophils, basophils.

Poultry are the captive birds, which are maintained on the natural habitat for the utility of the human beings. They are mainly maintained for egg, meat and feathers. There are two types of poultry i.e. terrestrial and aquatic poultry birds. There are about 15 species of terrestrial bird and 4 species of aquatic poultry birds present in India². Chickens (*Gallus gallus domesticus*) are most common terrestrial poultry bird, a subspecies of the red jungle fowl. Human keep chickens primarily for the source of food and less for the pet. White Embden geese is a breed of domestic goose. Domestic geese are the waterfowl of the family Anatidae, that kept by humans as poultry for their meat, eggs, and down feather since ancient times³.

II. MATERIALS AND METHODS

Ii.1 study area

The experiment was performed on white Embden geese (*Anser anser domesticus*) and vanaraja (*Gallus gallus domesticus*) being reared at Centurion University campus, BBSR, Odisha, India under the captive breeding.

Ii.2 blood collection

The blood samples were collected aseptically from the meta tarsal vein of the geese and chicken by the veterinary doctor using the 26 gauge needle and immediately one drop of blood was taken on a clean grease free slide (Borosil glass works Ltd. 1101, Crescenzo, G block, MCA club, Bandra Kurla complex, Bandra(E), Mumbai 400051, India. Ground edge, Measuring size 76mm long, 26mm wide and 1mm thickness) at one end immediately after collection.

Ii.3 preparation of slide

Another slide having a uniform edge was placed just in the drop of blood at an angle of 45° to the first and moved to touch the blood. After this, the spreader slide was moved gently over the smear slide without interruption till the end to have a uniform and smooth blood smear on the slide. Then the slide was air dried for 10 minutes and fixed with methanol (Sisco Research Laboratories pvt.Ltd. Plant site 1:D 88/2, MIDC, Turbhe, 400705, New Mumbai, India.) The slides were stained

with Giemsa stain (Hi Mediab Laboratories Pvt. Ltd.B/4 6, M.I.D.C.,Dindori,Nashik,MH.) prepared from Giemsa liquid and water, then the slides were kept for 40 minutes inside the coupling chamber. After this the slides were taken out from the staining rack and washed under a slow running tap water. From each specimen 10 slides were prepared and kept ready for processing and observation. The stained slides were then dried and observed under the microscope (BLISCO, INDIA, An ISO 9001:2008 Certified Co.) using 10X, 40X and 100X objectives and photomicrography after observation under the microscope. The cellular sizes were determined using ocular micrometer (10X) and stage micrometer.

Ii.4 morph metrical parameters

The morph metrical parameters like cell length, cell breadth, cell area, nuclear length, nuclear breadth, nuclear area and N/C ratio were calculated using the Microsoft Excel 2007 worksheet. For this experiment, 10 smeared slide were selected and dimension of different blood cells were calculated using Microscope.

Ii.5 statistical analysis

Data analysis was performed in Microsoft Excel 2007 by using t test: two sample assuming equal variance. Difference was classified as significant at $p < 0.05$. Cell area, nuclear area, n/c ratio were calculated using mathematical formula. The cellular area and nuclear area of elliptical erythrocytes were calculated using the formula $\pi \times LD/2 \times SD/2$ (LD = Longer diameter of the cell, SD = Shorter diameter of the cell). The area of rounded blood cell (leucocyte) were calculated by using the formula πr^2 . (r = Radius of circular cell, r = diameter/2). N/C ratio of erythrocyte and lymphocyte was calculated by nuclear area/cell area.

III. RESULTS AND DISCUSSION

Iii.1 Erythrocyte

Erythrocyte is the most important carrier of oxygen and carbon dioxide, its surface area to size ratio is a determining factor in the exchange of these gases with the tissue. Birds have elliptical body which is more efficient than a spherical one of the same volume. The high rates of metabolism of birds demand greater efficiency of gaseous exchange than that of many vertebrates. Aquatic birds have higher metabolic rate than the other non aquatic bird, so it reflects in the size of erythrocyte⁴. Birds have nucleated RBCs to make their homeostasis better. Nucleated RBCs enable them utilize water better so that aquatic bird have larger nuclei than the others. Nucleated RBCs help the aquatic bird capable of surviving in osmotic stress. The erythrocytes of both the chicken and geese were found oval to elliptical in shape under the compound microscope (**Fig.III.7**)(**Fig.III.8**). The mean size of

erythrocyte(Fig.III.1) of geese was $15.34\pm 0.22\mu\text{m}$ in length, $13.85\pm 0.19\mu\text{m}$ in width and $128.10\pm 1.86\mu\text{m}^2$ in cell area. The mean size of erythrocyte of chicken was $13.85\pm 0.19\mu\text{m}$ in length, $9.01\pm 0.20\mu\text{m}$ in width and $98.03\pm 2.54\mu\text{m}^2$ in cell area. The nucleus was oval or elliptical in shape and located at the center of the erythrocyte. The mean size of nucleus in erythrocyte of geese was $9.16\pm 0.24\mu\text{m}$ in length, 5.81 ± 0.21 in width and 41.82 ± 1.95 in nuclear area. The mean size of nucleus in erythrocyte of chicken was 8.34 ± 0.26 in length, 5.66 ± 0.12 in width and 36.99 ± 1.11 in nuclear area. The mean size of N/C ratio of geese was 0.32 ± 0.01 and chicken was 0.38 ± 0.01 (Table 1). Cytoplasm generally stained uniformly orange- pink with Giemsa stain. In the t-test analysis of comparison between aquatic and terrestrial bird, the result obtained that, the cell length, cell breadth and cell area values of erythrocytes were significant at $p<0.001$. The value of nuclear length and N/C ratio were significant at $p<0.01$. The nuclear breadth didn't reflect any significant value. The values of nuclear area was significantly differ at $p<0.05$.]

Table 1: Average value of Erythrocyte in relation to Geese and Chicken

Blood cell Parameters		Geese (Aquatic bird)	Range	Chicken(Terrestrial bird)	Range	P Value
Erythrocyte	Cell length	15.34 ± 0.22	14.15-16.39	13.85 ± 0.19	13.41-14.9	5.38E-05
	Cell breadth	10.63 ± 0.11	10.29-11.17	9.01 ± 0.20	8.19-10.43	1.05E-06
	Cell area	128.10 ± 1.86	120.35-137.24	98.03 ± 2.54	86.26-115.89	9.47E-09
	Nuclear length	9.16 ± 0.24	8.19-10.43	8.34 ± 0.26	7.45-9.68	0.018968
	Nuclear breadth	5.81 ± 0.21	4.47-6.70	5.66 ± 0.12	5.21-5.96	0.277993
	Nuclear area	41.82 ± 1.95	31.37-48.79	36.99 ± 1.11	30.49-41.82	0.022699
	N/C Ratio	0.32 ± 0.01	0.26-0.39	0.38 ± 0.01	0.26-0.44	0.010259

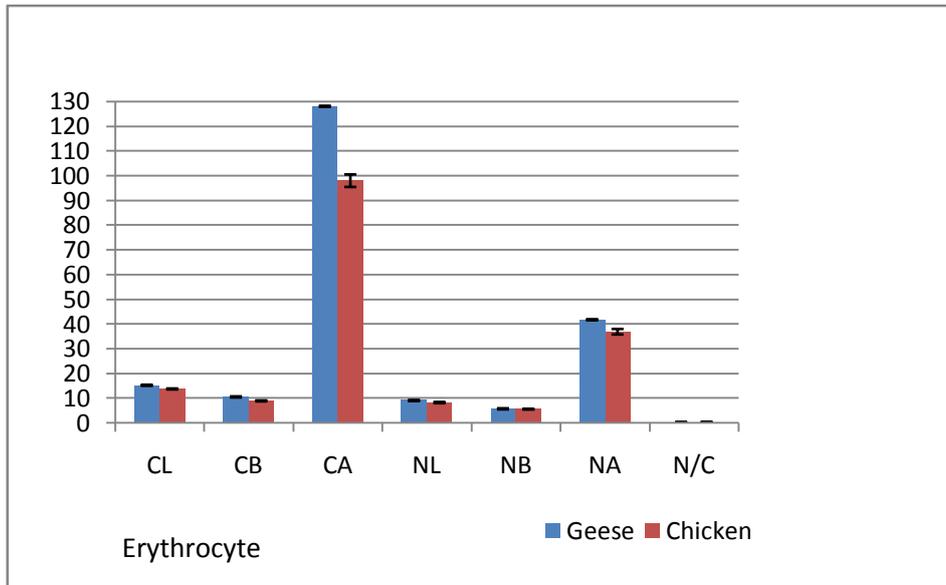


Fig.III.1 Comparison of erythrocytes of geese and chicken.

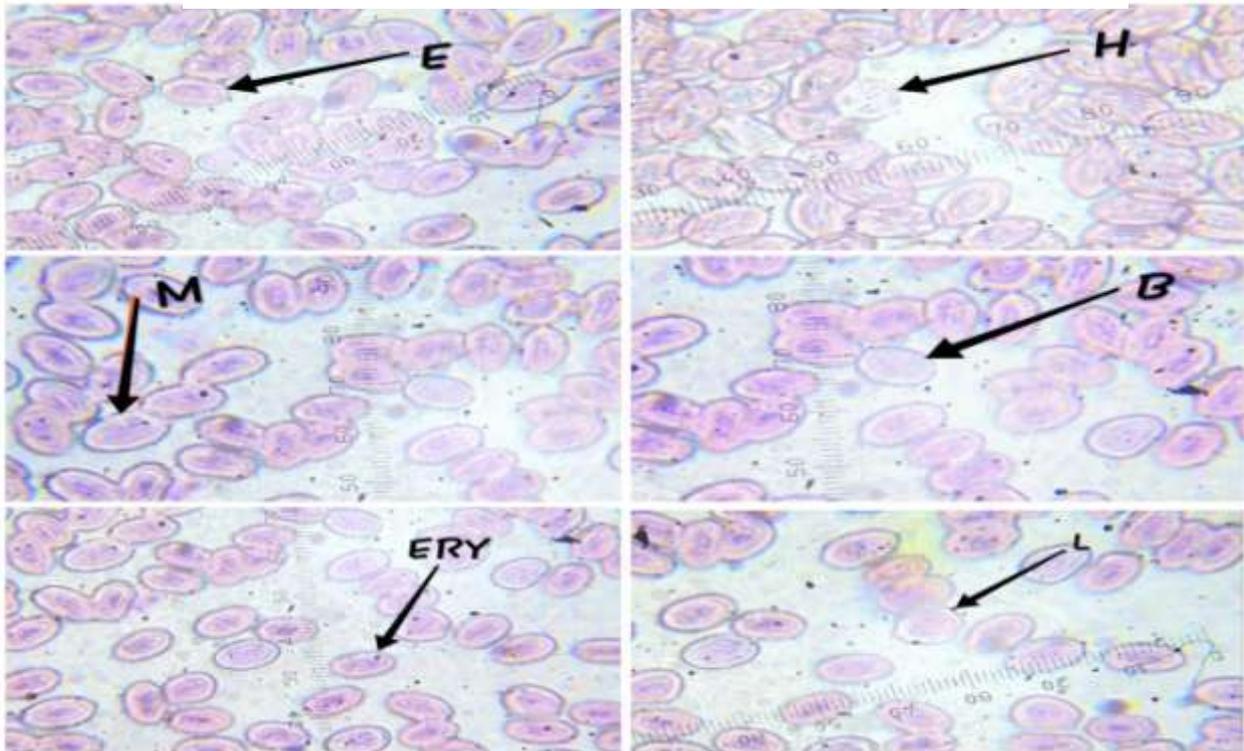


Fig.III.7 Blood cells of gesse; ERY- Erythrocyte, L- Lymphocyte, M-Monocyte,B-Basophil, E-Eosinophil, H-Heterophil.

Iii.2 Lymphocyte

Birds have higher size of lymphocyte than the other species. They have rounded in shape with densely stained nucleus(Fig.III.7)(Fig.III.8). Aquatic birds have higher lymphocyte size due to nutritional status and ambient temperature⁵. The lymphocyte size may be increase due to the prolonged effect of stress factor(Fig.III.2).This study resulted that the mean size of lymphocyte on

geese was $13.78 \pm 0.33 \mu\text{m}$ in length, $11.24 \pm 0.34 \mu\text{m}$ in width, $149.92 \pm 7.18 \mu\text{m}^2$ in cell area but chicken was $10.57 \pm 0.39 \mu\text{m}$ in length, $8.04 \pm 0.46 \mu\text{m}$ in width and $88.96 \pm 6.48 \mu\text{m}^2$ in cell area. The nucleus of the lymphocyte was rounded in shape. The nuclear size on lymphocyte of geese was $8.34 \pm 0.26 \mu\text{m}$ in length, $6.25 \pm 0.25 \mu\text{m}$ in width, $55.15 \pm 3.51 \mu\text{m}^2$ in nuclear area but chicken was $7.89 \pm 0.37 \mu\text{m}$ in length, $5.84 \pm 0.44 \mu\text{m}$ in width and $28.15 \pm 4.06 \mu\text{m}^2$ in nuclear area (Table 2). The N/C ratio in geese was 0.37 ± 0.03 and chicken was 0.32 ± 0.05 . In the t-test analysis of comparison between aquatic and terrestrial bird, the result obtained that the cell length, cell breadth, cell area and nuclear area of lymphocyte significantly differ at $p < 0.001$, but nuclear length, nuclear breadth and N/C ratio didn't reflect any significant value.

iii.3 Monocyte

Monocytes are the largest leukocytes found in the peripheral blood films. These have an abundant amount of cytoplasm compared to lymphocyte. The cytoplasm generally stains darker than the other lymphocyte (Fig.III.7)(Fig.III.8). Monocyte is cells of the innate immune system which perform important functions, including inflammatory cytokine production and phagocytosis, and participate in pathogen defence⁶. This study showed that aquatic birds have higher monocyte level than the non aquatic due to the effect of a pool-aquatic exercise⁷ (Fig.III.3). The effect of exercise can increase the phenotype, function and persistent changes in monocyte. The result showed that the mean size of monocyte on geese was $14.15 \pm 0.22 \mu\text{m}$ in length, $9.90 \pm 0.24 \mu\text{m}$ in width, $157.63 \pm 4.93 \mu\text{m}^2$ in cell area but chicken was $11.62 \pm 0.37 \mu\text{m}$ in length, $9.05 \pm 0.41 \mu\text{m}$ in width, $107.00 \pm 6.89 \mu\text{m}^2$ in cell area (Table 2). Comparison between aquatic and terrestrial, the result revealed that cell length and cell area significantly varies at $p < 0.001$ but cell breadth significantly differ at $p < 0.05$ with respect to habitat.

iii.4 Basophil

Avian basophils have round to oval, non-lobed nuclei that are often hidden by cytoplasmic granules (Fig.III.7)(Fig.III.8). Basophils appear in inflammatory reactions particularly that cause allergic symptoms. Aquatic birds have larger size of basophil (Fig.III.4) that may be the symptoms of allergic reaction because basophil found in unusually high numbers at sites of ectoparasite infections⁸. Basophil size of geese was $13.26 \pm 0.46 \mu\text{m}$ in length, $10.35 \pm 0.32 \mu\text{m}$ in width, $139.59 \pm 9.50 \mu\text{m}^2$ in cell area but the size of basophil on chicken was $9.98 \pm 0.44 \mu\text{m}$ in length, $8.19 \pm 0.31 \mu\text{m}$ in width and $79.64 \pm 6.93 \mu\text{m}^2$ in cell area (Table 2). In the t-test analysis of comparison between different habitat showed that, cell length, cell breadth and cell area of basophil significantly differ at $p < 0.001$.

Iii.5 Eosinophil

Avian eosinophils are round granulocytes and contain distinct round to oval cytoplasmic granules that lack the central refractile body(Fig.III.7)(Fig.III.8). Eosinophils are atype of WBC which is fighting against any disease. Higher rate of eosinophil may be the symptoms of inflammatory reactions i.e. eosinophilia. High intake of fat diet can causes the depletion of eosinophil size which showed on the nonaquatic bird⁹(Fig.III.5). The size of eosinophil on geese was14.08±0.28µm in length, 9.61±0.20µm in width, 159.19±6.19µm² in cell area but chicken was 13.85±0.19µm in length, 9.16±0.33in width and 151.01±4.40 in cell area(Table 2). Comparison between aquatic and terrestrial resulted that, cell length, cell breadth and cell area of eosinophil didn't show any significant value with respect to habitat.

Iii.6 Heterophil

Heterophils are the most abundant granulocyte in avian species. There nucleus is lobed containing fewer lobes than mammalian neutrophils¹⁰. The nuclear chromatin contains heavy chromatin clumping, the cytoplasm of normal mature heterophils is colorless and nonvaculated (Fig.III.7)(Fig.III.8). In the nonaquatic bird like chicken, diet and genetic line affect the heterophil activity, gene expression and enhance disease resistance. Aquatic birds have higher corticosterone diet that causes the lower heterophil size¹¹(Fig.III.6). Heterophil size in geese was 13.85±0.35µm in length, 10.74±0.47µm in width, 151.62±7.69µm²in cell area but chicken was 15.04±0.34µm in length, 11.92±0.34µm in width and 178.63±8.17µm²in cell area(Table 2). In the t-test analysis of comparison between different habitats showed that, cell length of heterophil reflected significantly at p<0.01.cell breadth and cell area significantly differ at p<0.05 with respect to habitat.

Table 2: Average value of Leukocyte in relation to Geese and Chicken

Blood cell Parameters		Geese(aquatic bird)	Range	Chicken(terrestrial bird)	Range	P Value
Lymphocyte	Cell length	13.78±0.33	11.92-14.9	10.57±0.39	8.94-11.92	4.21E-06
	Cell breadth	11.24±0.34	9.68-12.66	8.04±0.46	5.96-9.68	1.51E-05
	Cell area	149.92±7.18	111.53-174.27	88.96±6.48	62.74-111.53	3.07E-06
	Nuclear length	8.34±0.26	7.45-9.68	7.89±0.37	5.96-8.94	0.170955
	Nuclear breadth	6.25±0.25	5.21-8.19	5.84±0.44	3.72-7.74	0.211278
	Nuclear area	55.15±3.51	43.56-73.63	28.15±4.06	10.89-47.12	4.45E-05

	N/C Ratio	0.37±0.03	0.25-0.49	0.32±0.05	0.17-0.69	0.184094
Monocyte	Cell Length	14.15±0.22	13.41-14.9	11.62±0.37	10.43-13.41	7.67E-06
	Cell breadth	9.90±0.24	8.94-11.17	9.05±0.41	7.45-11.17	0.048191
	Cell area	157.63±4.93	141.16-174.27	107.00±6.89	85.39-141.16	6.02E-06
Basophil	Cell Length	13.26±0.46	10.43-14.9	9.98±0.44	7.45-11.92	4.06E-05
	Cell breadth	10.35±0.32	8.19-11.92	8.19±0.31	6.70-9.68	7.23E-05
	Cell area	139.59±9.50	85.39-174.27	79.64±6.93	43.56-111.53	3.77E-05
Eosinophil	Cell Length	14.08±0.28	12.66-14.9	13.85±0.19	13.41-14.9	0.262635
	Cell breadth	9.61±0.20	8.94-10.43	9.16±0.33	7.45-11.17	0.134935
	Cell area	159.19±6.19	125.91-174.27	151.01±4.40	141.16-174.27	0.251898
Heterophil	Cell Length	13.85±0.35	11.92-15.64	15.04±0.34	13.41-16.39	0.013713
	Cell breadth	10.74±0.47	8.94-12.96	11.92±0.34	10.43-13.41	0.030313
	Cell area	151.62±7.69	111.53-192.14	178.63±8.17	141.16-210.87	0.030313

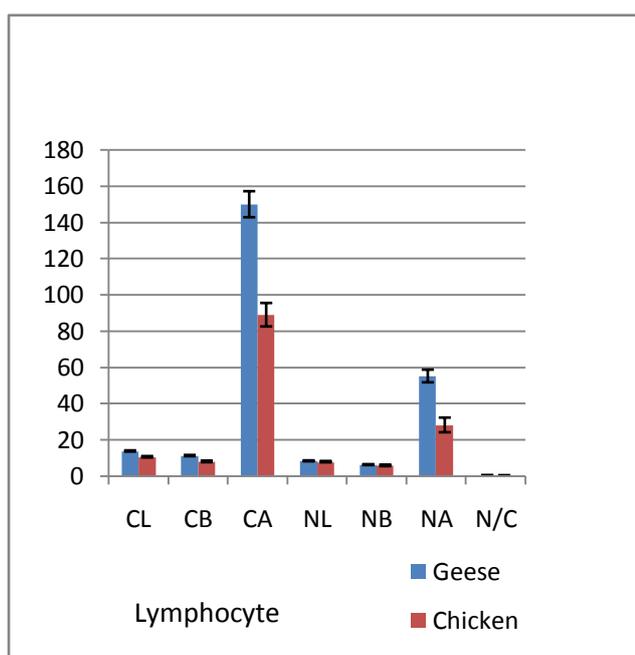


Fig.III.2 Comparison of lymphocyte of geese and chicken.

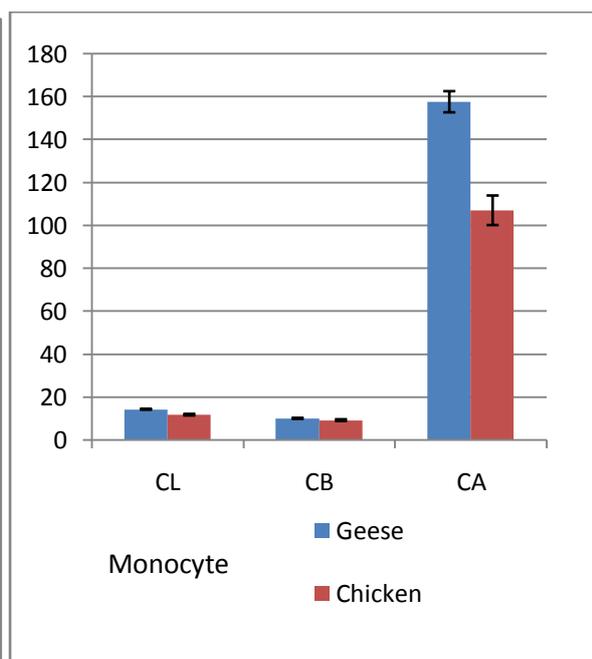


Fig.III.3 Comparison of monocyte of geese and chicken.

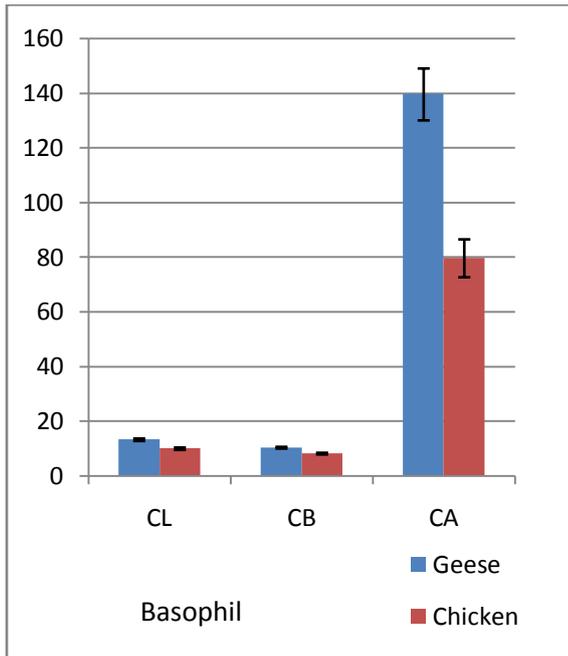


Fig.III.4 Comparison of Basophil of geese and chicken.

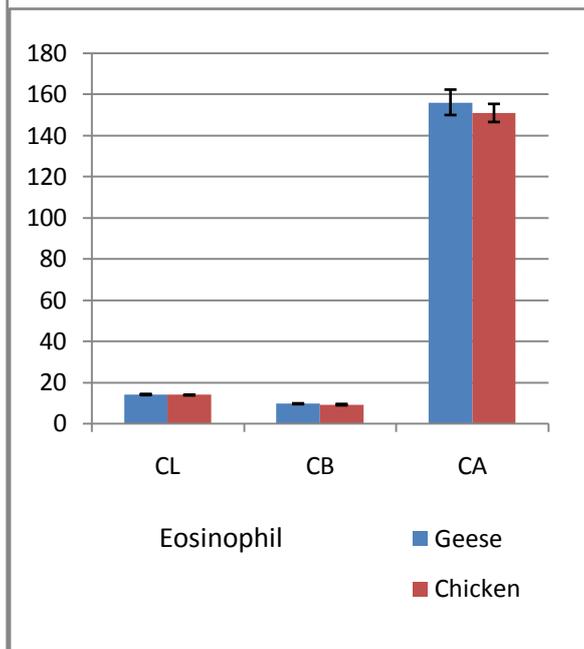


Fig.III.5 Comparison of Eosinophil of geese and chicken.

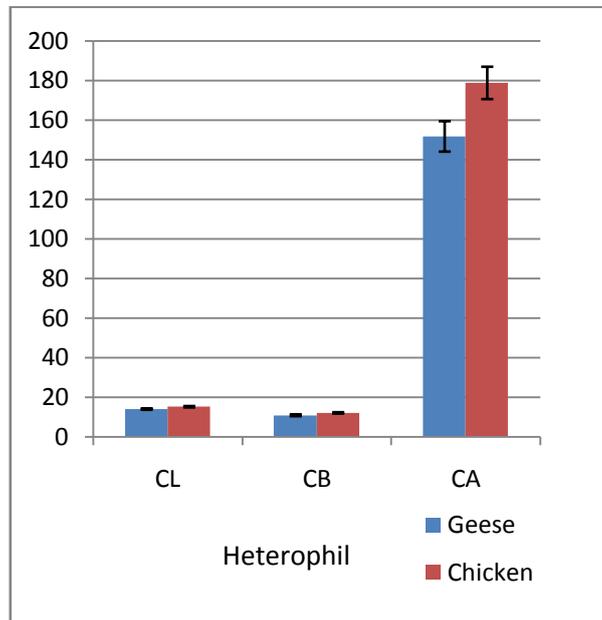


Fig.III.6 Comparison of Heterophil of geese and chicken.

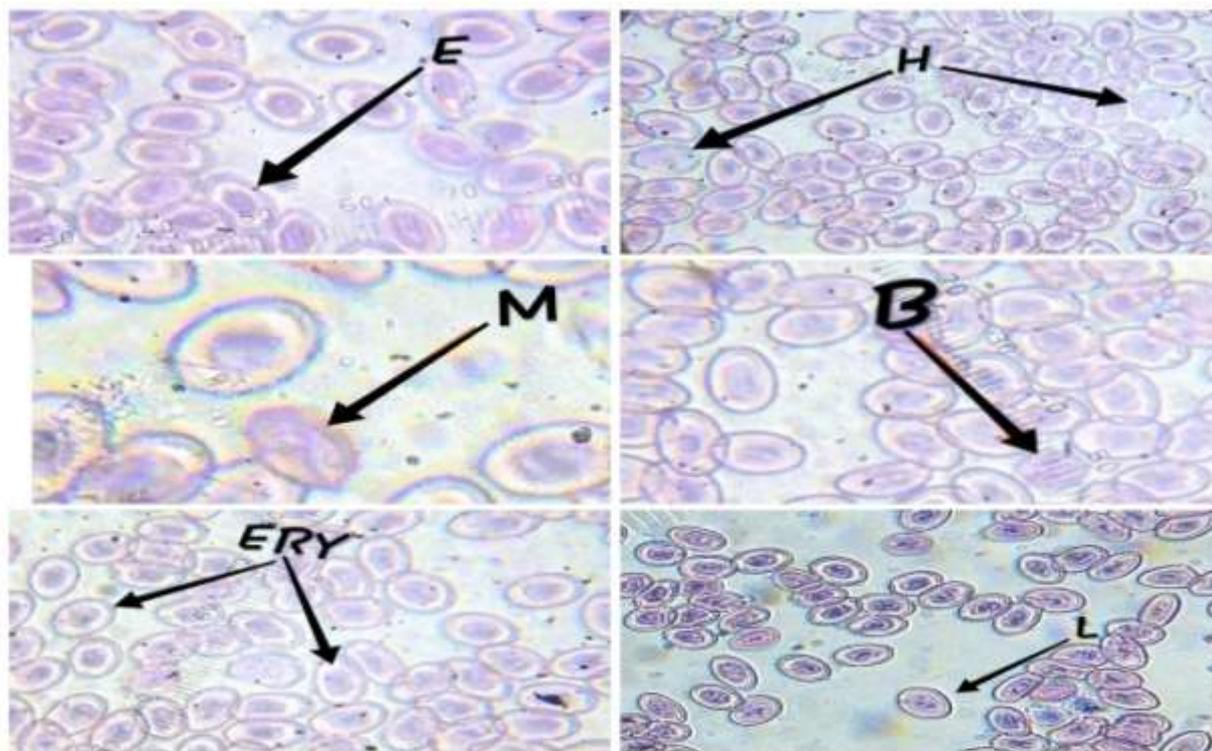


Fig.III.8 Blood cells of chicken; ERY- Erythrocyte, L- Lymphocyte, M-Monocyte,B-Basophil, E-Eosinophil, H-Heterophil.

IV.CONCLUSION

This study resulted that Aquatic poultry bird have higher size of blood cell except heterophil than the nonaquatic. Avian species have six types of blood cell i.e. Erythrocyte, Lymphocyte, Monocyte, Eosinophil, Basophil, Heterophil. Erythrocytes are mainly nucleated and elliptical or oval in shape . The lymphocytes are oval in shape with rounded nucleus. The monocytes are larger leucocyte and abundant in the peripheral blood cell. Eosinophil and Heterophil were rounded in shape with eccentrically placed lobulated nucleus. Basophils were largest, round to oval, non-lobed nuclei that are often hidden by cytoplasmic granules.The blood cell of gesse were larger in size due to high rate of metabolisim, nutritional status and ambient temperature, aquatic-exercise, inflammatory reactions. But higher corticosterone diet caused the lower heterophil size in the aquatic poultry bird.

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