

Comparative and Morphological Appearance of Blood Cell in Some Ducks and Fowls

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Abstract The blood samples were collected from the 20 numbers for 10 samples of fowls namely *Gallus domesticus* and *Gallus gallus domesticus* and 10 samples of ducks namely *Anas platyrhynchos* and *Anser cygnoides*. The blood samples of ducks were collected from Thongwa environs and the samples of fowls were collected from Theinphyu market. Yangon Region. The study period was from June 2015 to Dec 2016. Stained blood smears using wedge smear technique and Leishman stain was prepared. Blood cells were examined by X 1000. Cells types were identified based on well shape and mean size, nucleus shape and mean size in diameter and staining properties. The morphological and cytochemical features of erythrocytes and leucocytes thrombocytes of studied species were compared and discussed.

Keywords Blood cell shape and size, Nucleus shape and mean size in diameter

1. Introduction

In all vertebrates the blood comprise (1) a nearly colorless plasma, (2) white blood cell or leucocytes of several kinds, (3) red cells or erythrocytes. Colored by the contained hemoglobin which serve to transport oxygen and (4) smaller cells, the platelets, or thrombocytes. The plasma carries dissolved foods, wastes, internal secretions, and some gases (Storer and Usinger, 1979). Blood is a specialized body fluid in animals that delivers necessary substances such as nutrient and oxygen to the cells and transports metabolic waste products away from those same cells (Albers and Sanderson, 1996). Haematological and blood chemistry values can be measured easily and are useful in determining the health on several condition of birds (Siegel, 1968).

The blood contains a fluid plasma in which blood cells called corpuscles are suspended unattached to one another. Plasma is about 55 percent and corpuscles are 45 percent (Jordon, 2004). Red blood corpuscles (erythrocytes) contain iron, water, various inorganic salts and haemoglobin; they carry oxygen and help in removing carbon dioxide. There are two main types of white blood corpuscles or leucocytes, according to the shape of nuclei and presence or absence of granules in their cytoplasm. They are granulocytes and non-granular leucocytes. Granulocytes or granular leucocytes have numerous granules in cytoplasm and the

nucleus is segmented into two or more lobes, they are of three types, basophils, eosinophils and neutrophils. Non-granular leucocytes may or may not have small granular of neutral nature but the nucleus is never segmented into lobes. These leucocytes are lymphocytes and monocytes (Jordon, 2004).

In vertebrates, except mammals, the blood has thrombocytes or spindle cells, they are small in size with clear cytoplasm and an oval or rounded nucleus, and they help in clotting of blood (Jordon, 2004). Blood cell types and their morphology form an important part of disease diagnosis in groups of animals. By analyzing blood cell characteristics, diseases status can be identified (Anderson, 2008). In the warm-blooded birds and mammals the blood by differentials distribution between the internal organs and the body surface, serves to maintain the temperature of the entire body within close limit. Finally, the blood is the defense mechanism against foreign organisms having therefore a major role in maintain normal health and opposing the effects of infection (Store and Usinger, 1979).

The consumption of healthy and cleaning meats including fowls and ducks is important for health all over the world. Nowadays, the poultry farms occurred some infection of virus such as avian influenza. Thus, the good and healthy poultry farms may be breed in Myanmar. Expecting to get the normal hematological value and parameter from the studied species.

2. Objectives

This study was conducted to determine and compare the morphology of blood cells in some fowls and ducks.

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Therefore, this study is based on the following goals.

1. to examine composition of blood cell types and their morphology in the studied species
2. to determine and measure the mean diameter of the blood cells and their nuclei in the studied species
3. to compare the obtained data between the studied species

3. Materials and Methods

3.1. Study Area and Study Period

The ten blood samples from the two species of birds (duck), namely *Anas platyrhynchos* (Linnaeus, 1758) (Mallard) and *Anser cygnoides* (Linnaeus, 1758) (Goose) were collected from the Thongwa Township and other ten samples of the two species of birds (fowl), namely *Gallus domesticus* (Linnaeus, 1758) and *Gallus gallus domesticus* (Linnaeus, 1758) were collected from Theinphyu Market. Yangon Region.

3.2. Study Period

The study period lasted from June, 2015 to December, 2016.

3.3. Blood Sample Collection and Preparation

The right wing was extended fully and the medial aspect of the humeral area was sterilized with a cotton wool soaked in methylated spirit. Blood samples were collected from the brachial vein of the extended wing. The blood was drawn smoothly into a three ml syringe with an 18 gauge needle. The blood was collected in vacuum tube containing Ethylene Diamine Tetra Acetic acid (EDTA). Blood sample of 1 ml each was mixed into 3 ml of EDTA in a vacuum tube. The blood was mixed with the anticoagulant as soon as possible after collection by gentle rotation or inversion. EDTA treated samples are preferred for general haematological work. Basic experiments on haematology were done using routine thin blood film preparation and staining. The method was after Dacie and Lewis (1975) (Plate 3). The haematological analysis was done at laboratory of Department of Zoology, East Yangon University.

3.4. Thin Blood Film Preparation

Freshly drawn blood sample must be used in preparation of a thin blood film. Clean, grease-free slides with smooth unbroken ends were chosen. Place the slide on the counter or other horizontal surface. Place a medium sized drop of blood about 24 mm from the right end, midway between the edges of the slide. Hold this slide by pressing downward on the left hand. Take another slide, the spreader, in the right hand, holding it by its right end and place it in the center of the flat slide so that it forms an acute angle with the surface of the horizontal slide. Draw the spreader to the right until its end comes in contact with the drop of blood. Stop at this point and allow the blood to spread along the acute angle by

capillary action. Before the drop has spread to the edges of the horizontal slide, push spreader to the left in one continuous sweep. This pulls the thin blood film along. Smears should be thin and uniform, thickness is governed by (1) the size of the drop and (2) the angle of the spreader. An acute angle produces a thin smear. When the film is spread hasten drying with a little heat or current of air. Rapid drying of the film prevents crenation and fragmentation of the erythrocytes. Stain immediately and store in dust-free box to prevent flies, cockroaches, etc, from eating holes in the blood smear (Lewis, 1976).

3.5. Staining of Thin Blood Film

Leishman stain was carefully dropped onto the thin blood film so as to cover the whole thin blood film. The stain on the thin blood film was left for about 2 minutes. Then distilled water was added to the stain on the thin blood film to dilute. When a treat pipette, the diluted stain is then sucked up and down to mix it thoroughly. A correct dilution of the fluid will have a thin greenish scum. The diluted stain was allowed to act for 10 minutes after which it was washed off with distilled water, stand the slides for two stain an end to dry.

When dry, a liberal drop of immersion oil in the surface of the smear and proceed with the examination by oil immersion. Examined a large portion of the smear progressing from one edge to the middle and back again be sure to include the edges of the smear during this meandering. Continue this pattern until a representative area has been covered. The method was after Lewis (2000).

3.6. Formula of Leishman's Stain

Leishman's staining
Leishman's powder ----- 0.2 gm
Methanol ----- 100ml

Directions

0.2 gm of powdered dye is weighted out and transferred to a conical flask of 200-250 ml capacity. 100ml of methanol are added and the mixture is warmed to 50°C for 15 minutes with occasional shaking. The solution is filtered. It is then ready for use, but will improve on staining (Lewis, 2000).

3.7. Measuring the Blood Cells

Mature erythrocytes, leucocytes and thrombocytes and their nuclei were measured for diameter in each species of bird: and their mean diameter values in microns were calculated in each species followed after Murry (1992).

Formula for calculation of standard deviation

$$\bar{x} = \frac{\sum x}{n}$$

$$\hat{s} = \sqrt{\frac{\sum (x - \bar{x})^2}{N - 1}}$$

\hat{s} = Standard deviation

x = Diameter of one blood cell

\bar{x} = Average diameter of blood cell

N = Total number of blood cell (Murry, 1992)

3.8. Identification of Birds and Blood Cells

Morphological identification of erythrocytes, leucocytes, and thrombocytes were followed after Schermer (1958) and Thein (1979). The classification of the studied birds was followed after Robson (2005).

4. Results

The characteristic of the morphology of the erythrocytes, leucocytes and platelets (thrombocytes) in ducks and fowls were described.

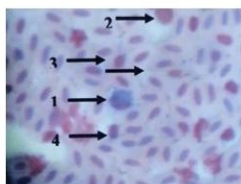
4.1. Systematic Position of the Studies Duck Species

Kingdom	: Animalia
Phylum	: Chordata
Class	: Aves
Order	: Anseriformes
Family	: Anatidae
Genus	: <i>Anas</i>
Species	: <i>A. platyrhynchos</i> (Linnaeus, 1758)
Common name	: Mallard
Genus	: <i>Anser</i>
Species	: <i>A. cygnoides</i> (Linnaeus, 1758)
Common name	: Goose

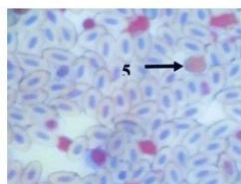
4.1.1. Blood Corpuscle of *Anas platyrhynchos*

Erythrocytes (RBC)

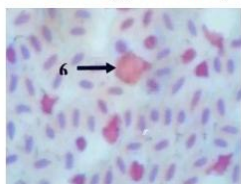
The red blood cells were flat oval disc with a biconvex nucleus which was oval in shape center. The mean diameter of the erythrocytes was $13.8 \pm 0.4 \mu\text{m}$ and the mean diameter of nucleus was $6.5 \pm 0.7 \mu\text{m}$ (Plate 1 and Table 1).



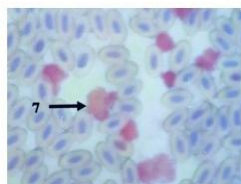
A. Monocyte (1), Lymphocyte (2)
Red blood cell (3), Thrombocyte (4)



B. Heterophil (5)



C. Eosinophil (6)



D. Basophil (7)

Plate 1. The blood cells of *Anas platyrhynchos*, Leishman stain (X 1000)

Leucocytes (WBC)

Granulocytes

The basophil had large granules and nucleus was twisted with three lobes constrictions. The mean diameter of basophil was $10.7 \pm 0.6 \mu\text{m}$. The eosinophil had large granules and the nucleus with two lobes connected by a

thread. The mean diameter of eosinophil was $11.5 \pm 1.0 \mu\text{m}$. The neutrophil had fine granules and the nucleus with only lobe. The mean diameter of neutrophil was $9.8 \pm 0.4 \mu\text{m}$ (Plate 1 and Table 1).

Non-granular leucocytes

The lymphocytes were round and had a nucleus with an indentation. The mean diameter of lymphocyte was $9.7 \pm 0.4 \mu\text{m}$. The monocyte was kidney shaped. It had more cytoplasm than lymphocytes. The mean diameter of monocytes was $9.8 \pm 0.4 \mu\text{m}$ (Plate 1 and Table 1).

Thrombocytes

The thrombocytes were small and oval in shaped; they had an oval shaped nucleus. The mean diameter of these blood cells was $6.8 \pm 0.4 \mu\text{m}$ and mean diameter of nucleus was $4.7 \pm 0.6 \mu\text{m}$ (Plate 1 and Table 1).

4.1.2. Blood Corpuscle of *Anser cygnoides*

Erythrocytes (RBC)

The red blood cells were flat oval disc with a biconvex nucleus which was oval in shape in the center. The mean diameter of the erythrocytes was $13.6 \pm 0.7 \mu\text{m}$ and the mean diameter of nucleus was $6.9 \pm 0.5 \mu\text{m}$ (Plate 2 and Table 1).

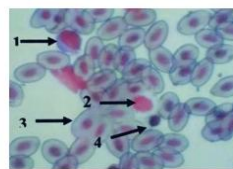
Leucocytes (WBC)

Granulocytes

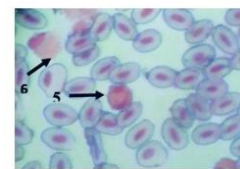
The basophil had large granules and nucleus was twisted with three lobes constrictions. The mean diameter of basophil was $11.7 \pm 0.4 \mu\text{m}$. The eosinophil had large granules and the nucleus with two lobes connected by a thread. The mean diameter of eosinophil was $11.6 \pm 0.7 \mu\text{m}$. The neutrophil had fine granules and the nucleus with only lobe. The mean diameter of neutrophil was $9.6 \pm 0.2 \mu\text{m}$ (Plate 2 and Table 1).

Non-granular leucocytes

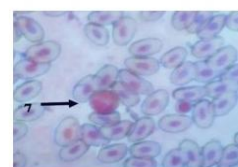
The lymphocytes were round and had a nucleus with an indentation. The mean diameter of lymphocyte was $9.6 \pm 0.2 \mu\text{m}$. The monocyte was kidney shaped. It had more cytoplasm than lymphocytes. The mean diameter of monocytes was $10.5 \pm 0.7 \mu\text{m}$ (Plate 2 and Table 1).



A. Monocyte (1), Lymphocyte (2)
Red blood cell (3), Thrombocyte (4)



B. Heterophil (5) Eosinophil (6)



C. Basophil (7)

Plate 2. The blood cells of *Anser cygnoides*, Leishman stain (X 1000)

Thrombocytes

The thrombocytes were small and oval in shaped. They had an oval shaped nucleus. The mean diameter of these blood cells was $6.8 \pm 0.4 \mu\text{m}$ and mean diameter of nucleus was $4.5 \pm 0.7 \mu\text{m}$ (Plate 2 and Table 1).

4.2. Systematic Position of the Studied Fowl Species

Phylum	: Chordata
Class	: Aves
Order	: Galliformes
Family	: Phasianidae
Genus	: <i>Gallus</i>
Species	: <i>Gallus domesticus</i> (Linnaeus, 1758)
Common name	: Chicken
Local name	: Tike-Kyet
Sub-species	: <i>Gallus gallus domesticus</i> (Linnaeus, 1758)
Common name	: Broiler-chicken
Local name	: Moy-meu-yac-kyet

4.2.1. Blood Corpuscles of *Gallus domesticus*

Erythrocytes (RBC)

The red blood cells were flat oval disc with a biconvex nucleus which was also oval in shape found to be in the center of the blood. The mean diameter of erythrocyte was $11.6 \mu\text{m}$ and the mean diameter of the nucleus was $4.7 \mu\text{m}$ (Plate 3 and Table 1).

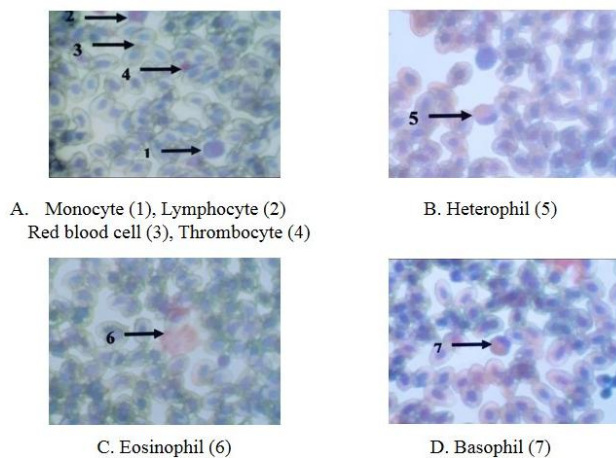


Plate 3. The blood cells of *Gallus domesticus*, Leishman stain (X 1000)

Leucocytes (WBC)

Granulocytes

The basophil had large granules and nucleus was twisted with two lobes constrictions. The mean diameter of basophils was $9.7 \mu\text{m}$.

The eosinophil had large granules. The nucleus had two lobes connected by a thread-like. The mean diameter of eosinophil was $8.7 \mu\text{m}$.

The neutrophil had fine granules. The nucleus had one lobe. The mean diameter of neutrophil was $7.7 \mu\text{m}$ (Plate 3 and Table 1).

Non-granular leucocytes

The lymphocytes were rounded had a nucleus with an indentation. The mean diameter of the lymphocyte was $8.7 \mu\text{m}$. The monocytes were large and rounded and its nucleus was bean shape. It had more cytoplasm than the lymphocytes. The mean diameter of monocyte was $11.7 \mu\text{m}$ (Plate 3 and Table 1).

Thrombocytes

The thrombocytes were small and irregular in shape, it had an oval shaped nucleus. The mean diameter of these blood cells were $5.7 \mu\text{m}$ and the mean diameter of nucleus was $4.7 \mu\text{m}$ (Plate 3 and Table 1).

4.2.2. Blood Corpuscles of *Gallus gallus domesticus*

Erythrocytes (RBC)

The red blood cells were flat oval disc with a biconvex nucleus which was also oval in shape found to be in the center of the blood. The mean diameter of erythrocyte was $11.5 \mu\text{m}$ and mean diameter of the nucleus was $8.7 \mu\text{m}$ (Plate 4 and Table 1).

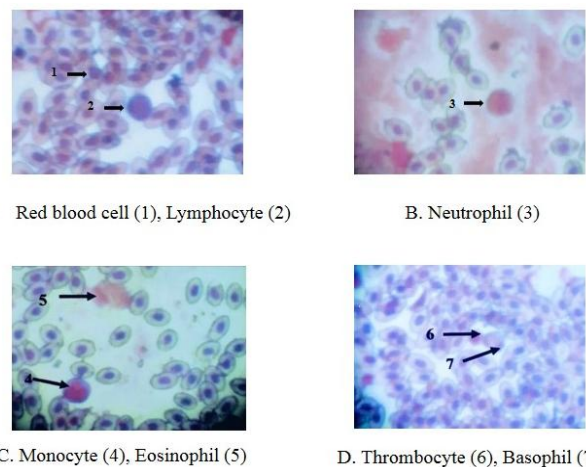


Plate 4. The blood cells of *Gallus gallus domesticus*, Leishman stain (X 1000)

Leucocytes (WBC)

Granulocytes

The basophil had large granules and the nucleus was twisted with two lobes constrictions. The mean diameter of basophil was $11.8 \mu\text{m}$. The eosinophil had large granules and the nucleus had two lobes connected by a thread-like. The mean diameter of eosinophil was $9.7 \mu\text{m}$. The neutrophil had fine granules. The nucleus had one lobe. The mean diameter of neutrophil was $10.7 \mu\text{m}$ (Plate 4 and Table 1).

Non-granular leucocytes

The lymphocytes were rounded and had a nucleus with an indentation. The mean diameter of the lymphocyte was $9.7 \mu\text{m}$.

The monocytes were large and rounded and its nucleus was bean shape. It had more cytoplasm than the lymphocytes. The mean diameter of monocyte was $10.8 \mu\text{m}$ (Plate 4 and Table 1).

Thrombocytes

The thrombocyte was small and irregular in shape, it had an oval shaped nucleus. The mean diameter of these blood

cells were 5.6 μm and the mean diameter of nucleus was 4.6 μm (Plate 4 and Table 1)

Table 1. Mean morphometric measurement of the blood cells diameter (μm) of the collected bird species

Sr. No.	Parameters	N	<i>Anas platyrhynchos</i>	<i>Anser cygnoides</i>	<i>Gallus domesticus</i>	<i>Gallus gallus domesticus</i>
1	Cell	10	13.8 \pm 0.4	13.6 \pm 0.7	11.6 \pm 0.7	11.5 \pm 0.8
	Nucleus	10	6.5 \pm 0.7	6.9 \pm 0.5	4.7 \pm 0.7	8.7 \pm 0.5
2	Basophil	10	10.7 \pm 0.6	11.7 \pm 0.4	9.7 \pm 0.7	11.8 \pm 0.4
	Eosinophil	10	11.5 \pm 1.0	11.6 \pm 0.7	8.7 \pm 0.5	9.7 \pm 0.5
	Neutrophil	10	9.8 \pm 0.4	9.6 \pm 0.2	7.7 \pm 0.5	10.7 \pm 0.5
	Lymphocyte	10	9.7 \pm 0.4	9.6 \pm 0.2	8.7 \pm 0.5	9.7 \pm 0.5
	Monocyte	10	9.8 \pm 0.4	10.5 \pm 0.7	11.7 \pm 0.5	10.8 \pm 0.4
3	Cell	10	6.8 \pm 0.4	6.8 \pm 0.4	5.7 \pm 0.6	5.6 \pm 0.7
	Nucleus	10	4.7 \pm 0.6	4.5 \pm 0.7	4.7 \pm 0.7	4.6 \pm 0.7

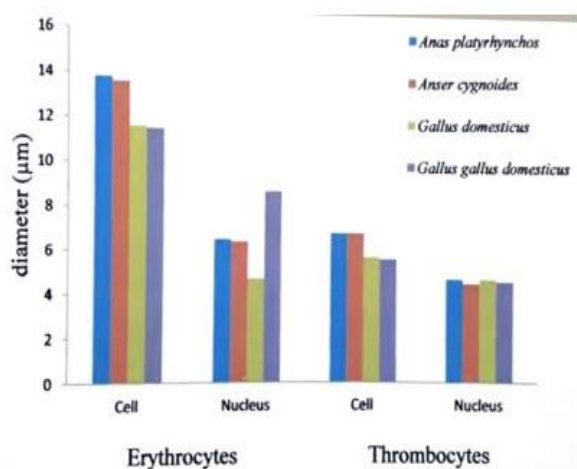


Figure 1. Comparison of mean diameter of red blood cells and thrombocytes (platelets) and their nuclei in the studied bird species

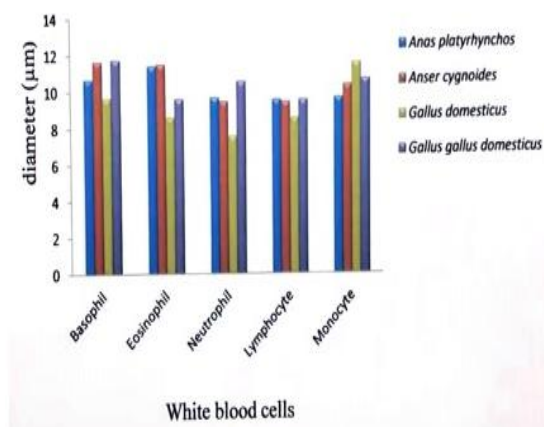


Figure 2. Comparison of mean diameter of white blood cells of their studied bird species

5. Discussions

In all vertebrates the blood comprises (1) nearly colorless plasma (2) white blood cells or leucocytes (3) red blood cells or erythrocytes (4) platelets or thrombocytes (Tibbo, 2004).

In this study, the blood sample of three species and one subspecies, *Anser cygnoides*, *Anas platyrhynchos*, *Gallus domesticus* and *Gallus gallus domesticus* were collected. From these samples, the characteristics of the morphology of the erythrocytes, leucocytes and platelets were described.

The red blood cells of the birds are oval and biconvex. The nuclei are also oval shape. In *Corvus splendens splendens*, *Lonchura ferruginosa atricapilla*, *Lonchura punctulata lineoventer*, *Streptopelia chinensis tigrina*, *Passer domesticus confucius*, *Porphyrio poliocephalus*, the largest red blood cells were observed in *Convus splendens splendens* (18 μm) and the smallest cells were found *Passer domesticus confucius* (6 μm) (Thein H., 1979).

The five studies of birds, *A.platyrhynchos*, *C. japonica*, *G.domesticus*, *G.gallus* and *G. g domesticus* have been domesticated in Myanmar for a long time. The mean diameter (nearly 12 μm) and nucleus of red blood cell and was equal (nearly 6 μm) in studied birds species (Khin, *et.al.*, 2014).

The mean diameter of red blood cell of *Conturnix japonica* (13 μm) was larger than the red blood cells of *Gallus gallus* (11 μm). The mean diameter of nucleus of red blood cells of *C.japonica* (7 μm) was also larger than *G.gallus* (6 μm) (Term paper, Group XXX VI 2013).

In this study, the largest mean diameter of red blood cells was found in *Anas platyrhynchos* (13.8 μm) and smallest in *Gallus gallus domesticus* (11.5 μm). And the largest mean of the diameter of nucleus of red blood cell was found in *Gallus gallus domesticus* (9 μm) and the smallest in *Gallus*

domesticus (5 μ m).

Among the birds, the largest basophiles were observed in *Porphyrio porphyrio policephalus* (12 μ m) and the smallest one were observed in *Lonchura punctulata lineoventer* (11 μ m) (Thein H., 1979).

The mean diameter of basophils (11 μ m), eosinophils (10 μ m) and neutrophils (10 μ m) were nearly equal size in studied species (Khin *et.al.*, 2014).

The mean diameter of basophil of *Coturnix japonica* (12 μ m) was larger than the basophil of *G.gallus* (11 μ m) (Term paper, Group XXX VI 2013).

In this study, the greatest mean diameter of basophils was observed in *Gallus gallus domesticus* (12 μ m) and the smallest mean diameter was studied in *Gallus domesticus* (10 μ m).

The largest mean diameter of eosinophil was observed in *Corvus splendens splendens* (11 μ m) and the smallest one was observed in *Passer domesticus confucius* (10 μ m) (Thein H., 1979).

The mean diameter of eosinophil of *Coturnix japonica* (12 μ m) was larger than the eosinophil of *G.gallus* (11 μ m) (Term paper, Group XXX VI 2013).

In this study, the largest mean diameter of eosinophil was found in *Anser cygnoides* (12 μ m) and the smallest one was found in *Gallus domesticus* (9 μ m).

The largest neutrophil were observed in *Porphyrio porphyria policephalus* (13 μ m) and the smallest one was observed in *Lonchura punctulata lineoventer* (10 μ m) (Thein H., 1979).

The mean diameter of neutrophil of *C.japonica* (11 μ m) was nearly equal with *G.gallus* (11 μ m) (Term paper, Group XXX VI 2013).

In this study, the neutrophils of the collected species were found to have very fine, numerous granules and the nucleus with only lobe. The largest mean diameter was studied in *Gallus gallus domesticus* (11 μ m) and the smallest one was studied in *Gallus domesticus* (8 μ m).

The largest mean diameter of lymphocyte was observed in *P.porohyrio policephalus* (13 μ m) and the smallest lymphocyte was observed in *Streptophila chinensis tigrina* (8 μ m) (Thein H., 1979).

The mean diameter of lymphocyte (11 μ m) and monocyte (11 μ m) were nearly equal in studied birds (Khin *et al.*, 2014).

The mean diameter of lymphocytes and monocytes were nearly equal in *G.gallus* and *C.japonica* (11 μ m) (Term paper, Group XXX VI 2013).

In this study, the largest mean diameter of lymphocyte was found in *Gallus gallus domesticus* (10 μ m) and the smallest lymphocyte was found in *Gallus domesticus* (9 μ m).

Monocytes are found in spleen, red bone marrow and are motile and phagocytic. The monocytes are largest cells along the white blood cells, they are also found in avian blood (Maxwell *et al.*, 1979).

In this study, the largest mean diameter of monocyte was studied in *G.domesticus* (12 μ m) and the smallest one was found in *Anas platyrhynchos* (10 μ m).

In vertebrates, except mammals, the blood has thrombocytes or spindle cells, they are small in size with clear cytoplasm and an oval or rounded nucleus, and they help in clotting of blood. Blood regulates and equalizes body temperature by carrying heat away from regions where it is produced (Jordon, 2004).

The average diameter of the thrombocytes is 4 μ m. The average diameter of the nucleus is 2 μ m. (Thein H., 1979).

The largest mean diameter (6 μ m) and nucleus (5 μ m) of thrombocytes was nearly equal in the studied species (Khin *et al.*, 2014).

The mean diameter of thrombocyte and was larger in *C.japonica* (6 μ m) with *G.gallus* (5 μ m) and the nucleus of thrombocyte was also larger in *C.japonica* (5 μ m) with *G.gallus* (4 μ m) (Term paper, Group XXX VI 2013).

In this study, the largest mean diameter of thrombocyte was found in *Anas platyrhynchos* (7 μ m) and the smallest one was found in *G.g.domesticus* (6 μ m). The mean diameters of nuclei of thrombocytes (5 μ m) were equal in studied birds. The freshly a clearly meats and eggs are important for consumption all over the world. Thus, the good and safely poultry breeding may be made for people.

6. Conclusions

In this study was conducted to determine normal hematological values of blood in the two species (*Anas platyrhynchos* and *Anser cygnoides*) of ducks and one species (*Gallus domesticus*) and one subspecies (*Gallus gallus domesticus*) of fowls. Blood species from 5 number of each species were used for the determination of hematological indices values. The morphological measures obtained in this study were red blood cells, three granular white blood cells consisting basophil, neutrophil and eosinophil and two non- granular white blood cells consisting lymphocytes and monocytes. However, there were specific variation to cell size between the studied species of ducks and fowls. The consumption of birds especially fowls, duck and their eggs are important for healthy diet. Thus the productions of cleaned and fleshy meats are essential for people. The good poultry farms must be breed in our country. In addition, the observing data from this work would also partially form the basis for further research on other species of blood cells.

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