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Morphometric Parameters of Erythrocytes in Juvenile Mandarin Ducks *Aix galericulata*

ABSTRACT

The morphometric parameters of erythrocytes in Mandarin ducks-*Aix galericulata* have never been subjected to study even though this species is common in aviculture. The aim of this study is to investigate the morphometric parameters of erythrocytes in juvenile Mandarin ducks. A total of 1500 erythrocytes obtained from thirty Mandarin ducks were analysed. The smears of the peripheral blood were stained using Diff-Quik® stain method, and the length and width of erythrocytes were measured using ImageJ program. The average length of the measured erythrocytes was 11.879 µm. The maximal measured length of the erythrocytes was 16.049 µm, and the minimal measured length was 5.668 µm. The average width of the measured erythrocytes was 6.160 µm. The maximal measured width of the erythrocytes was 10.115 µm and the minimal measured width was 3.750 µm. In conclusion, knowing morphometric parameters of Mandarin duck erythrocytes is the first step in evaluation process of erythrocytes' functional state and health condition of an individual.

INTRODUCTION

Several studies have described morphometric parameters and hematological characteristics of non-domestic *Anseriformes*, including: three species of geese (Williams & Trainer, 1971), canvasback duck *Aythya valisineria* (Kocan & Pitts, 1976), Pacific black duck *Anas superciliosa* (Mulley, 1979), Australian wood duck *Chenonetta jubata* (Mulley, 1980) and mallard *Anas platyrhynchos* (Driver, 1981; Fairbrother *et al.*, 1990; Fairbrother & O'Loughlin, 1990; Levengood *et al.*, 2000). These studies have described the morphological characteristics of hematological cells. Erythrocytes were ovoid cells with an ovoid nucleus composed of dense chromatin and homogeneous eosinophilic cytoplasm. The "ovoid" shape varies between species with some species having noticeably rounded cells whereas other species have a narrower, elongated shape. Mature avian erythrocytes are elliptical and have an elliptical, centrally positioned nucleus (Campbell, 2015). Avian mature erythrocytes are generally larger than mammalian erythrocytes but smaller than reptilian erythrocytes (Sturkie & Griminger, 1986).

In most species of birds, erythrocyte shape is relatively uniform, however, the shape of the red blood cells may be somewhat variable (Campbell, 2015). Variations in erythrocyte morphology occur in blood films from healthy birds as well as those with medical disorders. Therefore, atypical erythrocytes may vary in both size and shape. A significant change in the mean size of the erythrocyte is reflected in the mean corpuscular volume (MCV). A slight variation in the size of erythrocytes is considered in healthy birds. Flight less birds like the ostrich have the largest erythrocytes whereas the higher evolved good fliers, like



passerines, tend to have smaller erythrocytes (Maina, 1996; Welty, 1982). Erythrocyte subpopulations have been reported in ducks, in which larger erythrocytes (MCV, 308 fL/cell) most likely represent those most recently released from the hematopoietic tissue and smaller cells (MCV, 128 fL/cell) most likely represent the older, aging cells (Herbert *et al.*, 1989). Reticulocytes are the penultimate cell in the erythrocyte maturation series, and their presence in the peripheral blood of healthy birds suggests that the final stages of red-cell maturation occur in circulating blood. Reticulocytes tend to be smaller in size and less elongated compared to the mature erythrocyte (Campbell, 2015). Changes that typically occur in the size of avian erythrocytes include microcytosis, macrocytosis and anisocytosis. A greater degree of anisocytosis, however, usually is observed in birds with a regenerative anemia and is associated with polychromasia. Likewise, minor deviations from the normal shape of avian erythrocytes are considered to be normal in the peripheral blood of birds, but marked poikilocytosis may indicate erythrocytic dysgenesis (Campbell, 2015). The degree of variation in the size of erythrocytes anisocytosis can be scored from 1+ to 4+ based on the number of variable-sized erythrocytes in a monolayer field (Weiss, 1984). Food restriction anemia is also associated with a leukopenia, thrombocytopenia, abnormal erythrocyte shapes (marked poikilocytosis) and hypersegmentation of granulocytes (Maxwell *et al.*, 1991). Erythrocytes with variant morphology may be evident, depending on the cause of the anemia and the hematological response (Clark *et al.*, 2009). Microcytic, hypochromic, nonregenerative anemia is often associated with chronic inflammatory diseases in birds, especially those with an infectious etiology (Tell *et al.*, 2004). The presence of hemoparasites such as *Haemoproteus spp.* and *Plasmodium spp.* may alter the morphology of erythrocytes. The presence of the organism may distort the shape of the cell, enlarge the size of the cell, or displace the nucleus within the cell (Clark *et al.*, 2009).

The aim is to study the morphometric parameters of erythrocytes in juvenile Mandarin ducks *Aix galericulata*. The morphometric parameters of erythrocytes in Mandarin ducks have never been subjected to study even though this species is common in aviculture. Due to insufficient research and disadvantages of scientific papers from this scientific field, the knowledge about the morphometric parameters of erythrocytes in Mandarin ducks- *Aix galericulata* represent the beginning of hematological studies on the mandarin ducks. Although numerous quick and simple methods

for determination of qualitative and quantitative hematological values exist today, analysing the peripheral blood smears can be valuable when it comes to the species that haven't been yet studied extensively. The influence of different factors such as sex, mutation, age and reproductive status is proven to have impact on the hematological values of Mandarin ducks, as well as on the hematological values of other bird species. The influence of these factors on a species such as the Mandarin duck, that is still not considered domesticated, in aviculture, needs to be subjected to more detailed studies.

MATERIAL AND METHODS

The study was conducted in January 2018 and the blood smears from juvenile Mandarin ducks- *Aix galericulata* were taken from thirty Mandarin ducks which were six months old at the time. Blood samples from the normal (standard/wild colour), white and apricot Mandarin ducks were collected by puncturing the wing vein- *Vena ulnaris cutanea* using sterile needles and syringes and following the aseptic principles, according to authors (Janig *et al.*, 2017). Blood samples from all Mandarin ducks were taken during the process of National program of animal health strategy, which is determined by the Ministry of agriculture, forestry and water management Republic of Serbia. No bird was harmed during this process.

Total blood volume for birds is usually about 6-8 ml per 100g body mass (Sturkie *et al.*, 1986). We extracted 0.5 ml of blood from each Mandarin duck which would be about 0.2% of the total blood volume. Immediately after vein puncture and extracting 0,5 ml of each bird's blood, a few drops of blood without anticoagulants were transferred into microscope slides and the blood smears were made. These were dried promptly and stored until prepared for study in the laboratory. The smears were stained using Diff-Quik® stain method. After being dried, blood smears were observed under a microscope and the images were made. In this research 1500 morphometric parameters of erythrocytes were obtained from blood samples of thirty Mandarin ducks. According to the instructions of the authors (Janiga *et al.*, 2017) from each individual blood smear, from the thirty blood smears obtained, fifty erythrocytes were randomly chosen, whose morphometric parameters, or more precisely, the length and width of erythrocytes were calculated by using ImageJ, a public domain, Java-based image processing program developed at the National Institutes of Health.



RESULTS

This study is based on the morphometric parameters of 1500 measured erythrocytes which are obtained from blood smears of thirty Mandarin ducks. The length and width of each of the 1500 erythrocytes were determined and expressed in micrometers (μm) (Fig. 1 and 2). The average length of the measured erythrocytes was $11.879 \mu\text{m}$. The average width of the measured erythrocytes was $6.160 \mu\text{m}$ (Table 1). The maximal measured length of the erythrocytes was $16.049 \mu\text{m}$ whereas the minimal measured length was $5.668 \mu\text{m}$. The maximal measured width of the erythrocytes was $10.115 \mu\text{m}$ and the minimal measured width was $3.750 \mu\text{m}$ (Fig. 3). The average ratio of length to width of erythrocytes was 1.996.

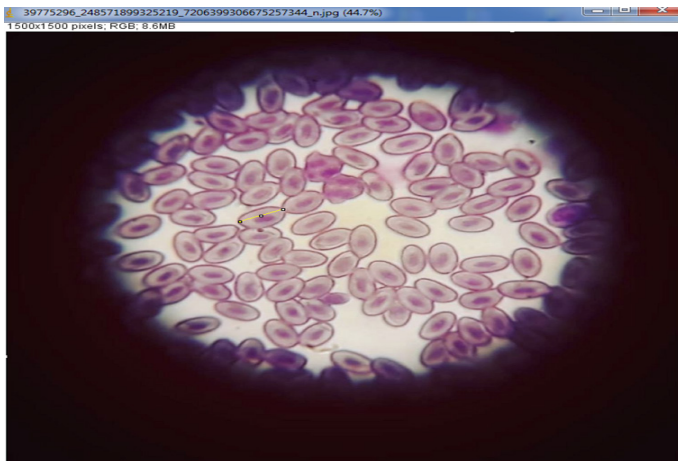


Figure 1 – Display of measuring length erythrocyte in Mandarin ducks using the ImageJ program. Following the random selection of each individual erythrocyte, using the ImageJ program, a line that measures the length of the erythrocyte, the value of measured erythrocyte length was obtained.



Figure 2 – Display of measuring width of singular erythrocyte in Mandarin ducks using the ImageJ program. Following the random selection of each individual erythrocyte, using the ImageJ program, a line that measures the width of the erythrocyte, the value of the measured erythrocyte width was obtained.

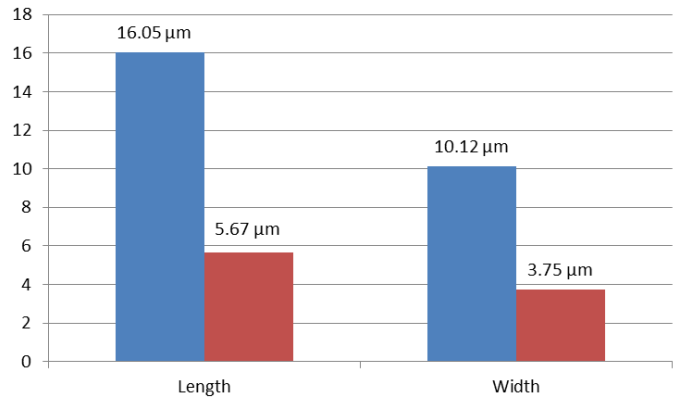


Figure 3 – Extreme (maximal and minimal) length and width of the measured erythrocytes of Mandarin duck's *Aix galericulata*.

Table 1 – Average values of morphometric parameters of Mandarin duck's *Aix galericulata* erythrocytes

Erythrocytes (Er) measured = 1500	μm
Average length (L)	11.879
Average width (W)	6.160
Average L/W	1.996

DISCUSSION

Autors Hartman & Lasser (1963) in their research of birds erythrocytes, have measured average ratio of length and width of erythrocyte. The highest value of average ratio of length and width was $2.11 \mu\text{m}$ and it was recorded forth species *Cardellinapusilla*, while the lowest value of average ratio of length and width was $1.62 \mu\text{m}$ and it was recorded forth species *Turdusplebejus* and *Thraupisepiscopus*. In this research, average value of this ratio in erythrocytes of Mandarin duck was $1.99 \mu\text{m}$.

According to Rusov (2002), the erythrocytes in peripheral blood smears have a $10.7\text{-}15.5 \mu\text{m}$ long and $2.64\text{-}2.25 \mu\text{m}$ wide diameter, depending on bird species. The average length of Mandarin ducks' erythrocytes determined in this study corresponds to the values that Rusov (2002) described. However, the average width of the measured erythrocytes was $6.160 \mu\text{m}$ and it is almost three times larger than what he described.

Avian erythrocytes vary in size depending on the species, but they generally range between $10.2\text{-}15.8 \mu\text{m}$ and $6.1\text{-}10.7 \mu\text{m}$ (Sturkie & Griminger, 1986). Their results correspond to the results of this study of morphometric parameters of erythrocytes in Mandarin ducks with the length of erythrocytes – $11.879 \mu\text{m}$ and width – $6.160 \mu\text{m}$.

According to Tadjalli *et al.* (2003) measures of adult *Coturnix quail* erythrocytes were $11.06 \pm 0.70 \mu\text{m}$ in



length and $6.80 \pm 0.67 \mu\text{m}$ in width in males and $11.40 \pm 0.63 \mu\text{m}$ in length and $6.73 \pm 0.45 \mu\text{m}$ in width in females. The results of this study are almost the same as the results of the study that Tadjalli *et al.* (2003) performed, although the blood smears weren't divided according to the ducks' sex. But according to Sturkie & Griminger, (1986) measures of adult *Coturnix quail* erythrocytes were $9.80 \pm 0.77 \mu\text{m}$ in length and $8.23 \pm 0.72 \mu\text{m}$ in width in males and $9.80 \pm 0.77 \mu\text{m}$ in length and $7.73 \pm 0.70 \mu\text{m}$ in width in females. When compared to the results in this study, it's clear that the erythrocytes in *Coturnix quail* (regardless of the sex of the quails) are significantly smaller in size than the erythrocytes in Mandarin ducks.

According to Biester & Schwarte (1955), the average length of chickens' erythrocytes is $12.2 \mu\text{m}$ and the average width is $7.3 \mu\text{m}$. When compared to the results of this study, the values are similar, as the average length of Mandarin ducks' erythrocytes is $11.879 \mu\text{m}$ and the average width of Mandarin ducks' erythrocytes is $6.160 \mu\text{m}$.

Comparison of birds with their ancestral relatives, the reptiles, shows how great the divergence is between these groups. According to Wintrobe (1961), reptilian erythrocytes range from $18.1\text{--}8.7 \mu\text{m}$ (turtle) to $23.2\text{--}12.1 \mu\text{m}$ (alligator). These differences indicate how far ahead on the metabolic scale the birds are and also Mandarin duck with average length of erythrocytes $11.87 \mu\text{m}$ and the average width erythrocytes $6.16 \mu\text{m}$.

Comparison of the erythrocytes of birds with other warm-blooded animals is interesting. In mammals their diameters range from $5\text{--}6 \mu\text{m}$ in horse, cow, pig, mouse and rat, to $7\text{--}7.4 \mu\text{m}$ in chimpanzee, woodchuck and llama (Wintrobe, 1961). This author's results compared to the results of this study show that birds have larger erythrocytes than mammals.

Seasonal variations, age and species affect the erythrocyte parameters of healthy birds, especially wildlife (Harper & Lowe, 1998; Hauptmanova *et al.*, 2006; Rehder *et al.*, 1982; Schmidt *et al.*, 2007; Shave & Howard, 1976). These authors' claims can be compared to the results of this study as all the birds were of the same age and under the same climate conditions. The above-mentioned factors can be linked to the determined minimal and maximal length ($5.66 \mu\text{m}$ and $16.04 \mu\text{m}$) and minimal and maximal width ($10.11 \mu\text{m}$ and $3.75 \mu\text{m}$) of erythrocytes in Mandarin ducks.

Age can be a cause of variation in the normal hemogram of healthy ducklings. Difference in hematologic parameters likely including erythrocytes

associated with the high metabolic rate of young rapidly growing ducks that are consuming diets higher in protein and carbohydrates (Kocan & Pitts, 1976). Some studies have shown that averages for erythrocyte parameters tend to be higher in the winter and pre-nesting period in adult ducks and geese regardless of gender compared to the post-nesting period and fall (Williams & Trainer, 1971; Shave & Howard, 1976). Although the results of this study represent the first data on the erythrocytes of the Mandarin ducks, they can be compared to these authors' results as the blood samples were taken during the winter season, two months before the reproductive season for these birds started.

CONCLUSION

Erythrocytes, as the most numerous blood cells, represent one of the basic indicators of health condition in birds. Making of blood smears is a simple and practical method used to determine various pathological processes inside birds' organism, caused by erythrocytes size change. Knowing morphometric parameters of Mandarin duck erythrocytes is the first step in the evaluation process of erythrocytes' functional state and health condition of an individual.

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