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Research Article

A Study on Sex-Based Hematology and Biochemistry of Lesser Whistling Duck (*Dendrocygna javanica*)

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Abstract

Background and Objective: Hematological and biochemical data are important physiological aspects that can be used as an initial strategy to assess, monitor and manage wildlife. Hematological and biochemical profiles are influenced by age, sex, postprandial, season, climatic conditions, reproductive status and diurnal changes. This study aimed to measure the hematological and biochemical parameters of *Dendrocygna javanica* based on its sex. **Materials and Methods:** A total of 24 *Dendrocygna javanica* (12 male and 12 female) were captured from two different locations. Blood samples were taken using superficial metatarsal plantar. Meanwhile, the time lag between capture and blood sampling was 3 minutes to avoid physiological changes due to stress. The blood was taken as much as 1% of body weight using a 23 gauge needle. Samples were inputted to vacutainers, EDTA tubes, for hematological examination and non-additive vacutainer for biochemical examination. Statistical analysis of hematological and biochemical parameters between sexes was performed using a t-test with a 95% confidence level. **Results:** The calculation results of erythrocytes, mean corpuscular volume (MCV), eosinophils, heterophils, lymphocytes, monocytes and the H/L ratio showed significant differences between males and females. Aspartate aminotransferase (AST) and glucose in males were higher than females ($p < 0.05$). **Conclusion:** Hematological and biochemical parameters in males tend to be higher than females.

Key words: *Dendrocygna javanica*, mean corpuscular volume, eosinophil, heterophils, lymphocytes, monocytes, H/L ratio, aspartate aminotransferase, glucose

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Data from the directorate general of livestock and animal health at the Ministry of Agriculture indicates that meat production in 2016 was 3.4 million tons. Poultry had made a major contribution to the meat production at 65.26%. Although, there was an increase, there had been a decline in meat production in poultry compared to 2015. To offset the demand rate, in addition to increasing the productivity of existing livestock, it is necessary to explore animals that were expected to have the potential as alternatives for producing meat².

One of the wild ducks belonging to the *Anatidae* family in Indonesia is *Dendrocygna javanica* which lives in freshwater and bushes. Moreover, this *Dendrocygna javanica* has the potential to produce meat². Many people have hunted and caught this animal continuously in the North Sumatra region to be consumed or to be treated as a pet. If this activity is continuously carried out, then its population will gradually decrease and even become extinct.

Some studies on the morphology of *Dendrocygna javanica* have been conducted³. Hematological and biochemical data can be important physiological aspects that can be utilized as an initial strategy for assessing, monitoring and managing wildlife which were recognized as quite easy, economical, fast and practical⁴. Hematological studies were also important clues in assessing nutrition, health conditions, metabolism status, stress responses and phylogenetic relationships⁵.

The hematological profile is influenced by physiological (age, sex, postprandial, season, climatic conditions, reproductive status and diurnal changes), pathological and treatment factors⁶. A study conducted by Hauptmanova *et al.*⁷ concluded that RBC count, PCV, Hb and MCHC in male pheasant chickens were higher than those of female pheasant chickens. The same data were also found in a seel chickens⁸, local quails⁹, ducks¹⁰ and Japanese quails¹¹.

The biochemical reference of blood in wild animals is very important for detecting pathological conditions and evaluating nutritional status¹². Biochemical parameters of blood are influenced by age and sex¹³. Villegas *et al.*¹⁴ stated that the concentration of ALP in female *Geronticus eremita* was higher than that of the males. Court *et al.*¹⁵ found that the concentrations of ALP, triglycerides and calcium in female *Platalea leucorodia* were higher than males.

However, hematological and biochemical data in *Dendrocygna javanica* have not been known yet. Thus, this study aimed to measure hematological parameters including hemoglobin, packed cell volume (PCV), total erythrocytes,

total leukocytes, differential leukocyte counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin count (MCHC). This study also aimed to investigate the biochemistry of blood of Lesser whistling duck including ALP (alkaline phosphatase), AST (aspartate aminotransferase), total protein, albumin, cholesterol, triglycerides, blood urea nitrogen (BUN), uric acid, creatinine and glucose.

MATERIALS AND METHODS

The study was conducted from June to October 2019. The *Dendrocygna javanica* was obtained from two different locations, Perbaungan District, Serdang Bedagai Regency and Tanjung Pasir Village, Pangkalan Susu District, Langkat Regency, North Sumatra Province, Indonesia using bird control services. The ducks were hunted at night using bird traps. The blood samples were taken from 12 male and 12 female ducks. The ducks were weighed before the blood sampling. Blood was taken aseptically through superficial plantar vein metatarsal veins with the help of a veterinarian. The time lag between the capture and blood sampling was 3 min to avoid physiological changes due to stress¹⁶. The blood smear was made in conjunction with blood sampling. The volume of blood samples taken were as much as 1% of body weight or 3 mL using a needle measuring 23 gauge and 1 inch long. Blood samples were placed into the vacutainer containing EDTA for hematological examination and non-additive vacutainer for biochemical examination. After that, the samples were put into a thermo flask that contained ice with a temperature of 12-15°C. The examination later was immediately carried out.

Hematological parameters (total erythrocytes, hemoglobin, PCV, MCV, MCH, MCHC, total leukocyte count and differential leukocytes) were measured. Calculation of total erythrocyte, hemoglobin, PCV and total leukocyte was conducted according to Campbell¹⁷. Meanwhile, the calculation of differential leukocytes was done using Wright-Giemsa's staining¹⁸. The slides were waited till dry before being observed using a microscope with 1000× magnification. MCV, MCH and MHCH values were determined using Campbell's formula¹⁷.

Biochemical parameters including alkaline phosphatase (ALP), aspartate aminotransferase (AST), total protein, albumin, cholesterol, triglycerides, blood urea nitrogen (BUN), uric acid, creatinine and glucose were measured. The method used to measure biochemical parameters is presented in Table 1.

The t-test was used to compare hematological and biochemical parameters between sexes. The data was statistically analyzed using SPSS version 20, with 95% confidence level.

Table 1: Method of analysis

Parameters (Unit)	Method (test kit/reagent)
ALP (U L ⁻¹)	Kinetic method (IFCC)
AST (U L ⁻¹)	Kinetic method (IFCC)
Total protein (g dL ⁻¹)	Biuret reagent
Albumin (g dL ⁻¹)	Bromocresol green
Cholesterol (mg dL ⁻¹)	CHOD-PAP
Triglycerida (mg dL ⁻¹)	GPO-PAP
BUN (mg dL ⁻¹)	Colorimetric
Uric acid (mg dL ⁻¹)	TBHBA
Creatinine (mg dL ⁻¹)	Mod Jaffe
Glucose (mg dL ⁻¹)	GOD-PAP

RESULTS

The hematologic value of *Dendrocygna javanica* was obtained and presented in Table 2. The results indicate that there were no significant differences in PCV, Hb, MCHC, leukocyte count and basophils between sexes. However, there were significant differences in the count of erythrocytes, MCV, MCH, eosinophil, heterophile and lymphocyte, monocyte and H/L ratio between sexes. The results of measurements of adult *Dendrocygna javanica* biochemical parameters are presented in Table 3. These results indicate that there was no significant difference in ALP, total protein, albumin, cholesterol, triglycerides, BUN, creatinine and gout. However, there were significant differences between AST and glucose between sexes.

DISCUSSION

This study found that erythrocyte value in males was higher than females. Similar results were also found for *Numida meleagris*¹⁹ and *Cairina moschata*²⁰. According to Schmidt *et al.*²¹, the value of erythrocytes was influenced by age, sex, hormonal, environment and disease. Tadjalli *et al.*¹⁰ stated that sex had a significant effect on erythrocyte, PCV, platelets and MCHC. Furthermore, Hebert *et al.*²² reported that, in general, the male erythrocyte value was higher compared to females. According to Khan and Zafar²³, high estrogen levels caused decrease in the value of erythrocyte and hemoglobin. Vander *et al.*²⁴ insisted that the hormone testosterone stimulates erythropoietin release which acts to stimulate the proliferation and maturation of erythrocytes in the bone marrow while estrogen inhibits erythropoietin. Moreover, the sampling time and reproductive cycle phases also affected all hematological parameters in both male and female⁷.

PCV and hemoglobin values did not show significant differences although there was a tendency for females to get higher values than males. Similar results were also found for

Table 2: Haematological values of male and female *Dendrocygna javanica*

Parameters	Sex	
	Male (n = 12)	Female (n = 12)
	Mean ± SD	Mean ± SD
Erythrocytes (× 10 ⁶ μL ⁻¹)	3.00 ± 0.69*	2.31 ± 0.27
PCV (%)	28.00 ± 6.14	33.66 ± 2.60
Hb (g dL ⁻¹)	9.10 ± 2.37	10.76 ± 3.67
MCV (fl)	94.62 ± 9.15*	146.24 ± 7.74
MCH (pg)	30.68 ± 4.32*	45.67 ± 11.24
MCHC (pg)	32.29 ± 1.80	31.39 ± 8.44
Leucocytes (× 10 ³ μL ⁻¹)	81.06 ± 8.33	79.86 ± 22.15
Basophils (%)	3.66 ± 0.49	3.16 ± 1.64
Eosinophils (%)	4.83 ± 1.26*	2.83 ± 1.40
Heterophils (%)	30.66 ± 2.6*	26.66 ± 3.44
Lymphocytes (%)	63.33 ± 3.44*	54.00 ± 0.85
Monocytes (%)	6.33 ± 0.49*	5.33 ± 0.98
Heterophils/Lymphocytes (H/L)	0.56 ± 0.05*	0.42 ± 0.07

*p<0.05

Table 3: Biochemical values of male and female *Dendrocygna javanica*

Parameters	Sex	
	Male (n = 12)	Female (n = 12)
	Mean ± SD	Mean ± SD
ALP (U L ⁻¹)	141.23 ± 10.05	145.17 ± 14.54
AST (U L ⁻¹)	31.26 ± 2.41*	15.33 ± 1.02
Total protein (g dL ⁻¹)	3.51 ± 0.68	3.74 ± 0.48
Albumin (g dL ⁻¹)	1.43 ± 0.14	1.40 ± 0.08
Cholesterol (mg dL ⁻¹)	319.83 ± 5.76	312.00 ± 14.38
Triglycerides (mg dL ⁻¹)	67.35 ± 5.35	69.13 ± 6.95
BUN (mg dL ⁻¹)	26.83 ± 1.74	25.5 ± 3.75
Creatinine (mg dL ⁻¹)	0.15 ± 0.01	0.14 ± 0.24
Uric acid (mg dL ⁻¹)	11.58 ± 156.4	12.82 ± 2.55
Glucose (mg dL ⁻¹)	214.75 ± 9.76*	176.25 ± 15.01

*p<0.05

*Meleagris gallopavo*²¹ and *Anas superciliosa*²⁵. Moreover, MCV and MCH values in females were also higher than males. This happened, because the calculation of MCV and MCH was influenced by the total erythrocyte. The low erythrocyte had an impact on increasing the MCV and MCH values. MCHC in males was slightly higher than females. However, MCHC values did not show significant differences between genders. The same result was also found for *Cygnus olor*²⁶ and *Gracula religiosa*²⁷.

Leukocyte and basophile percentage between sexes also did not show a significant difference. However, there was a tendency for males to get a higher percentage than females. Leukocyte variability was influenced by various factors such as season, individual, stress, immune system condition and sampling time^{28,29}.

Additionally, the percentage of lymphocytes in males was higher than females. Similar results were also found in roosters based on a study conducted by Kadaknath and Rajasri⁸. Different types of leukocytes are influenced by age, hormones

and stress³⁰. Increasing the percentage of lymphocytes in males might be caused by stress since males were more aggressive when captured and when the blood was taken for the sample. Lymphocytosis was characterized by an increase in epinephrine which occurred due to physical and emotional stress³¹. In most birds, lymphocytes were the second most leukocytes after heterophils. However, it can be inferred that both females and males had higher lymphocytes than other leukocytes to be said as lymphocytic species.

The percentage of eosinophil, heterophils, monocytes and H/L ratio in males was higher than females. The H/L ratio was an indicator to measure stress levels in birds³². This difference might be related to the stress response that occurs during the capture, treatment and blood sampling. Similar results were also found in chickens treated with high temperatures³³.

Increased ALP enzyme activity in birds was a consequence of increased osteoblast activity and hepatocyte damage⁶. In this study, there were no significant differences in ALP between sexes even though females tended to have higher ALP than males. Similar results were also found in cherry valley duck³⁴ and swan¹². However, Gee *et al.*³⁵ obtained different results based on several studies on *Anatidae*.

Increased AST levels might occur due to impaired liver or muscle function. This study obtained the results that AST levels in males were higher than females. The same data were also found in turkeys³⁶ and Vanaraja³⁷. An increase in AST might occur due to excessive muscle movement during the process of capturing, handling and blood sampling²⁶.

The total protein is an important parameter for evaluating nutritional status in birds. It is also useful for detecting infectious diseases³⁸. In this study, total protein in males tended to be lower than females. The increase in total protein was influenced by the estrogen³⁹. However, there were no significant differences between sexes. Similar results were also found in local ducks⁴⁰ and Khaki Campbell ducks⁴¹. In general, total protein in ducks was influenced by genotype, age and nutrition⁴²⁻⁴⁴. Albumin levels also did not show significant differences but were still within the normal range of 40-50%⁴⁵.

Balasz *et al.*⁴⁶ reported that BUN concentrations in males were quite high compared to those found in other birds ranging from 7-14.9 mg dL⁻¹. This might be due to the lack of availability of natural food. The highest BUN value ever reported was in buzzard birds which reached 50 mg dL⁻¹ due to the absence of food supply and changes in eating habits^{46,47}.

According to Lumeij⁴⁸, the creatinine value in *Dendrocygna javanica* was slightly lower than the reference value for birds, in general, which was 0.30-1.90 mg dL⁻¹. Previous studies^{16,26,49} showed that uric acid levels between males and females were still within the normal range in most *Anatidae*, which ranged from 5-12 mg dL⁻¹. Lumeij⁴⁸

reported that cholesterol levels in males and females were higher than the reference value for non-migratory *Anatidae* which was 104-244 mg dL⁻¹. However, cholesterol value of *Dendrocygna javanica* was lower compared to *Anas clypeata* and *Anas crecca*¹⁶. Moreover, triglyceride levels did not show a significant difference between males and females. Similar results were also found in cherry valley duck³⁴.

Male blood glucose levels were higher than females. The same results were also found in *Anas platyrhynchos*⁵⁰. Increased glucose levels were influenced by several factors such as postprandial conditions, increased metabolic activity and stress^{51,52}. Stress might be triggered during the capture, handling, or blood sampling. According to Lumeij⁵³, the normal reference value of avian blood glucose ranged from 11-25 m mol L⁻¹, equivalent to 198-450 mg dL⁻¹. Thus, glucose levels in males (214, 75 mg dL⁻¹) were still within the limits of normal reference values which were lower in females (176, 25). Elarabany¹⁶ stated that glucose levels of male and female *Dendrocygna javanica* were quite low because glucose levels in *Anatidae* ranged from 232-369 mg dL⁻¹. This might be due to a lack of food availability. Furthermore, Calabuig *et al.*¹² found that glucose levels were related to the quantitative and qualitative composition of the food.

CONCLUSION

Hematologic parameters such as erythrocyte, MCV, eosinophil, heterophils, lymphocytes, monocytes and H/L ratio showed differences between males and females. Biochemical parameters such as AST and glucose in males were higher than females ($p < 0.05$). Hematological and biochemical parameters in males tended to be higher than females.

SIGNIFICANCE STATEMENT

This study found differences in several hematological and biochemical parameters between male and female *Dendrocygna javanica*. This study was intended to be helpful for future researchers to determine physiological reference values that can be used to detect pathological conditions and nutritional status of *Dendrocygna javanica*. Compared to the same species or with other species, *Dendrocygna javanica* can be used for conservation purposes in the future.

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