ORIGINAL ARTICLE

Blood cell characteristic, hematological and serum biochemistry values of Painted Stork (*Mycteria leucocephala*)

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Abstract

Aengwanich, W., Tanomtong, A., Pattanarungson, R., and Simaraks, S.
Blood cell characteristic, hematological and serum biochemistry values of Painted Stork (*Mycteria leucocephala*)
Songklanakarin J. Sci. Technol., 2002, 24(3) : 473-479

Blood cell characteristics, hematological and serum biochemistry values were examined in 10 mature healthy Painted Storks (*Mycteria leucocephala*) (5 males and 5 females) from Khow Khoew Open Zoo, Chonburi province, Thailand. The morphology of erythrocytes, leukocytes and thrombocytes were similar to those of other avian species, but oval shaped granules of heterophils were less stained by Giemsa-Wright solution. Erythrocytes, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total white blood cells, percentage of leukocytes (lymphocyte, heterophil, monocyte, eosinophil, basophil), thrombocytes, serum glucose, serum creatinine, serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase values of Painted Storks were 3.24 ± 0.82 10^6cells / μl, 0.82 10^6cells /μl, 17.67 ± 1.56 g/dl, 42.40 ± 3.57 %, 139.47 + 38.70 fl, 58.56 ± 18.24 g/dl,

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Received, 15 November 2001 Accepted, 24 May 2002

41.76 ± 2.90 pg, 3.88 ± 2.60 10^4cells /µl, 76.10 ± 10.28 %, 10.60 ± 9.49 %, 0.00 ± 0.00 %, 12.00 ± 8.54 %, 1.00 ± 1.33 %, and 1.29 ± 0.4210^{4}cells /µl, 270.50 ± 42.85 mg/dl, 6.19 ± 1.70 mg/dl, 249.47 ± 42.19 IU/L and 124.29 ± 8.81 IU/L, respectively. Total erythrocytes and mean corpuscular volume (MCV) differed significantly between male and female Painted Stork (P < 0.05).

Key words : blood cell characteristics, hematology, serum biochemistry, Painted Stork (*Mycteria leucocephala*)

บทคัดย่อ

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ลักษณะเซลล์เม็ดเลือด ค่าโลหิตวิทยาและชีวเคมีโลหิตของนกกาบบัว (*Mycteria leucocephala*)

ว. สงขลานครินทร์ วทท. 2545 24(3) : 473-479

การศึกษาลักษณะเม็ดเลือด ค่าทางโลหิตวิทยาและชีวะเคมีโลหิตของนกกาบบัวโตเต็มวัยที่มีสุขภาพดีจำนวน 10 ตัว (เพศผู้และเพศเมียชนิดละ 5 ตัว) จากสวนสัตว์เปิดเขาเขียว จังหวัดชลบุรี ผลการศึกษาพบว่าลักษณะเม็ดเลือดของนกกาบบัวชนิดต่าง ๆ มีลักษณะเหมือนกับเม็ดเลือดของสัตว์ปีกทั่วไป แต่แกรนูลของเม็ดเลือดขาวชนิดเฮทเทอโรฟิลย้อมติดสีย้อมชนิด Giemsa - Wright น้อยกว่านกชนิดอื่น ค่าจำนวนเม็ดเลือดแดงทั้งหมด ค่าฮีโมโกลบิน ค่าเปอร์เซ็นต์เม็ดเลือดอัดแน่น ค่าเฉลี่ยปริมาตรเม็ดเลือดแดง ค่าเฉลี่ยฮีโมโกลบิน ค่าเฉลี่ยฮีโมโกลบินต่อ 1 เซลล์เม็ดเลือดแดง ค่าจำนวนเม็ดเลือดขาวทั้งหมด ค่าเปอร์เซ็นต์เม็ดเลือดขาว (ลิมโฟไซต์ เฮทเทอโรฟิล โมโนไซต์ อีโอซิโนฟิล และแบโซฟิล) ค่าเกล็ดเลือด ค่ากลูโคสในซีรั่ม ค่าครีเอตินีนในซีรั่ม ค่าเอนไซม์ serum glutamic oxaloacetic transaminase และ ค่าเอนไซม์ serum glutamic pyruvic transaminase ของนกกาบบัวที่วัดได้มีค่าเท่ากับ 3.24 ± 0.82 10^6cells /µl, 0.82 10^6cells /µl, 17.67 ± 1.56 g/dl, 42.40 ± 3.57 %, 139.47 + 38.70 fl, 58.56 ± 18.24 g/dl, 41.76 ± 2.90 pg, 3.88 ± 2.60 10^4 cells /µl, 76.10 ± 10.28 %, 10.60 ± 9.49 %, 0.00 ± 0.00 %, 12.00 ± 8.54 %, 1.00 ± 1.33 %, and 1.29 ± 0.4210^4 cells /µl, 270.50 ± 42.85 mg/dl, 6.19 ± 1.70 mg/dl, 249.47 ± 42.19 IU/L and 124.29 ± 8.81 IU/L ตามลำดับ และพบว่าค่าจำนวนเม็ดเลือดแดงทั้งหมดและค่าเฉลี่ยปริมาตรเม็ดเลือดแดงของนกกาบบัวเพศผู้และเพศเมียแตกต่างกันอย่างมีนัยสำคัญทางสถิติ(P<0.05)

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Panted Stork (*Mycteria leucocephala*) is a large and brightly colored wading bird of Indian sub- continent (Hvass, 1963), South-West China and parts of South East Asia and can be found on fresh lakes, marshes and paddy fields (King *et al*., 1975, Innes, 2000, and Coulter *et al*., 1989). Its classification in animal kingdom is phylum Chrodata, class Aves, order Ciconiiformes, family Ciconiidae (Perrins and Middlelon, 1985; Perrins, 1990; Thomson, 1964 and Pettinggill, 1956) and genus Mycteria (Lekagul and Crownin, 1974). This stork is slightly less than a meter a height (56 to 58 inches.) (Innes, 2001). It is white in color with black markings and delicate pink on the lower back (Gupte, 2001), black and white lesser than wing covert, and a black band across the breast (King *et al*., 1975 and Lekagul, 1970). Painted Stork had a large heavy yellow beak and orange-red head; the long legs are pinkish in color. Males and females look alike (King *et al*., 1975 and Perrins, 1990). This food is mainly fishes and frogs, which they get by wading into shallow

water. Its nesting season is during the monsoon in north India and November to January or February in south India. Nests are made of strikes usually built on trees or close to water, 3 - 5 eggs are laid and nesting period 7 - 18 weeks (Perrins, 1990). Both males and females take part in incubating and rearing the chicks (Gupte, 2001).

At present, Painted Storks are an endangered species. The purpose of this study was to establish blood cell characteristics, hematological and serum biochemistry values for Painted Storks. Knowledge of the characteristics and these values may be applied in physiological study, health diagnosis or in-depth study directed toward their conservation.

Materials and Methods

Blood specimen - Blood samples (4 ml) were obtained from the brachial vein (Campbell, 1995) by 23 gauge, 1.5 inches needle in 10 mature Painted Storks (5 males and 5 females) in Khow Khoew Open Zoo, Chonburi province, Thailand, then immediately transferred to 3 tubes; 1 ml into the first tube containing NaF for determination of serum glucose, 1 ml into the second tube containing EDTA for determination of hematological values and 2 ml into the third tube without anticoagulant for determination of other serum biochemistry (Ritchie, *et al*., 1994). The specimens were cooled to approximately 4°C, using icepacks and transferred to the laboratory.

Laboratory technique - The samples were collected approximately 12 hours prior to the preparation of blood smear by standard technique then processed with Giemsa-Wright staining. Hematological values for packed cell volume, hemoglobin concentration, red blood cell count and white blood cell count were determined by centrifuging, Cyanomethemoglobin method, unopette system and Natt and Herrick's method, respectively. The mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration calculated from the proceeding values were: packed cell volume, hemoglobin concentration and total red blood cell (Campbell, 1995). Serum biochemistry for glucose, creatinine, serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase were determined by enzymatic method, Jaff's Alkaline Picrate: end point and transminase method (Settasatial and Teerajettakul, 1992), respectively.

Statistical analysis - The results were given as mean $\pm$ SD, hematological and blood biochemistry values between males and females were compared by t - test (Daungjinda, 2001) using SAS system (SAS, 1996) and a level of significance set at $P< 0.05$.

Results and Discussion

The morphology of erythrocyte, white blood cell and thrombocyte of Painted Stork are showed in Figures 1 - 4.

This is the first record of hematological and serum biochemistry values of Painted Storks. Erythrocyte, hemoglobin, packed cell volume, blood indices (mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration), total white blood cell, percentage of lymphocyte, heterophil, monocyte, eosinophil, basophil, thrombocyte, serum glucose, creatinine, serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase values for painted storks were $3.24 \pm 0.82\ 10^6$ cells /µl, 17.67 ± 1.56 g/dl, 42.40 ± 3.57 %,

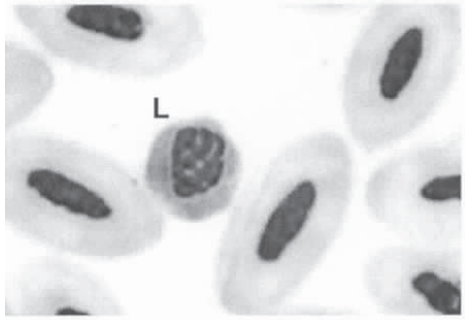


Figure 1. An immature lymphocyte (L) of *Mycteria leucocephala*, Giemsa - Wraight stain. (100X)

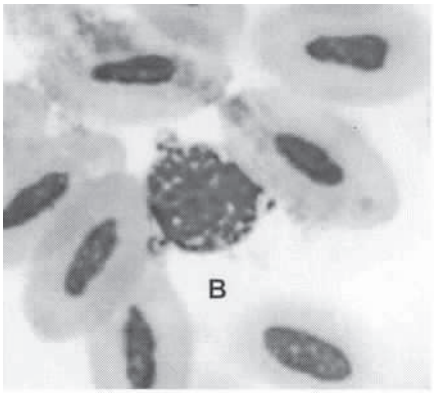


Figure 2. A Basophil (B) of *Mycteria leucocephala*, Giemsa - Wraight stain. (100X)

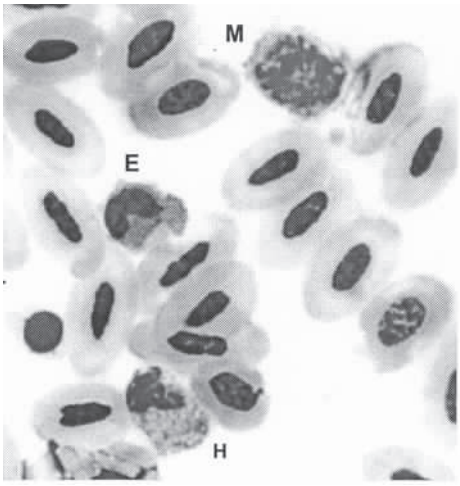
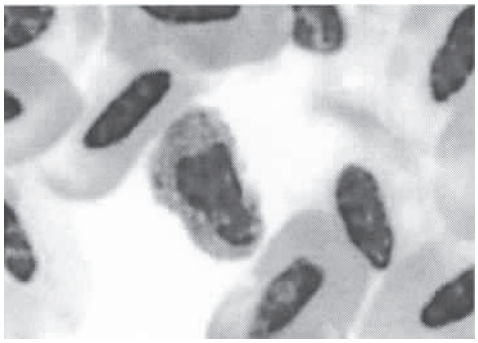
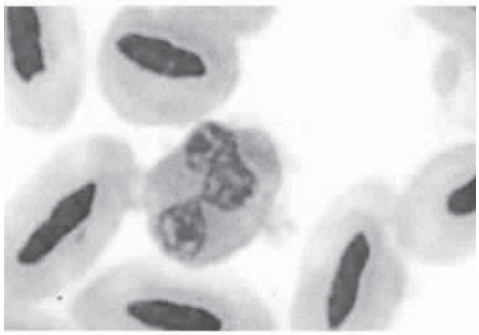


Figure 3. Monocyte(M), eosinophil (E), heterophil (H) and thrombocyte (T) of *Mycteria - leucocephala*, Giemsa-Wraight stain. (100X)

139.47 + 38.70 fl, 58.56 ± 18.24 g/dl, 41.76 ± 2.90 pg, 3.88 ± 2.60 10^4cells /µl, 76.10 ± 10.28 %, 10.60 ± 9.49 %, 0.00 ± 0.00 %, 12.00 ± 8.54 %, 1.00 ± 1.33 %, and 1.29 ± 0.4210^{4}cells / µl, 270.50 ± 42.85 mg/dl, 6.19 ± 1.70 mg/dl, 249.47 ± 42.19 IU/L and 124.29 ± 8.81 IU/L, respectively (Table 1). Comparison of hematological and serum biochemistry values of Painted Stork between males and females Painted Stork



A.



B.

Figure 4. An eosinophil (A and B) of *Mycteria - leucocephala*, Giemsa - Wraight stain. (100X)

found that the total erythrocytes of males were higher than that of females and mean corpuscular volume of females was significantly ($P< 0.05$) greater than that of males.

Matured erythrocytes of Painted Storks were homogeneous in size, shape, and color. They were typically oval shaped with a centrally positioned oval nucleus that contained dense and dark-staining chromatin. The heterophil was a single round to irregular cell with spindle to oval shaped cytoplasmic granules (Figure 3). Giemsa-Wright solution, stained these granulesonly weakly. The basophil was slightly smaller than the heterophil and the cytoplasm contained strongly basophilic granules. These cells were often easily identified on Giemsa-Wright stained blood smears by the presence of numerous, deeply basophilic, round cytoplasmic granules (Figure 2). Eosinophils were of non-typical shape with abundant amounts of cytoplasm containing numerous distinct oval to

Table 1. Hematological and blood biochemistry values of Painted Stork (*Mycteria leucocephala*).

Values	Males (n=5)	Females(n=5)	Total (n=10)	Range
Hematology				
Erythrocyte (10^6cells /µl)	3.79 ± 0.46*	2.68 ± 0.73	3.24 ± 0.82	2.42 - 4.06
Hemoglobin (g/dl)	17.84 ± 1.99	17.50 ± 1.21	17.67 ± 1.56	16.11 - 19.23
PCV (%)[1]	43.60 ± 3.36	41.20 ± 3.70	42.40 ± 3.57	38.83 - 45.97
MCV (fl)[2]	116.86 ± 20.56	162.08 ± 40.84	139.47 ± 38.7	100.77 - 178.17
MCH (g/dl)[3]	47.84 ± 9.39	69.28 ± 19.32	58.56 ± 18.24	40.32 - 76.80
MCHC (pg)[4]	40.98 ± 3.90	42.54 ± 1.47	41.76 ± 2.90	38.86 - 44.66
White blood cell (10^4cells /µl)	2.95 ± 2.75	4.81 ± 2.32	3.88 ± 2.60	1.28 - 6.48
Lymphocyte (%)	76.60 ± 9.99	75.60 ± 11.72	76.10 ± 10.28	65.82 - 86.38
Heterophil (%)	8.00 ± 5.87	13.20 ± 12.30	10.60 ± 9.49	1.11 - 20.09
Monocyte (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 - 0.00
Eosinophil (%)	14.00 ± 10.27	10.00 ± 6.96	12.00 ± 8.54	3.46 - 20.54
Basophil (%)	0.80 ± 1.10	1.20 ± 1.64	1.00 ± 1.33	0 - 2.33
Thrombocyte (10^4cells /µl)	1.16 ± 0.38	1.42 ± 0.46	1.29 ± 0.42	0.87 - 1.71
Blood biochemistry				
Glucose (mg/dl)	292.0 ± 38.34	249.0 ± 38.79	270.50 ± 42.85	227.65 ± 313.35
Creatinine (mg/dl)	6.68 ± 0.89	5.70 ± 2.26	6.19 ± 1.70	4.49 - 7.89
SGOT (IU/L)[5]	263.76 ± 37.32	235.18 ± 45.84	249.47 ± 42.19	207.28 - 291.66
SGPT (IU/L)[6]	124.28 ± 10.49	124.30 ± 8.04	124.29 ± 8.81	115.48 - 133.1

*** Within rows, values with no common superscript differ significantly (P< 0.05)**

[1, 2, 3, 4, 5, and 6] mean packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase, respectively.

round granules. The cytoplasmic granules of eosinophils were smaller than those of heterophils and typically stained brighter in the same blood film. The nucleus had 2 -3 lobules with contained dense and dark-staining chromatin. Lymphocytes constituted an average 75 % of the leukocytes seen in the normal clinically (Figures 3 and 4) Painted Storks evaluated in this study. The immature lymphocyte was a round cell in the blood film (Figure 1). The monocytes were the largest leukocyte found in the peripheral blood film (Figure 3). Thrombocytes were small oval to round cells; the nucleus was pyknotic and the cytoplasm colorless (Figure 3). The morphologic features of blood cells from Painted Stork, *Mycteria leucocephala* were similar to those of other species of avian.

Most hematological and blood biochemistry values of males and females Painted Storks were not different, but the erythrocyte number of males was significantly higher than that of females (P<0.05). Sturkie (1965) reported that numbers of erythrocytes were influenced by androgen. Besides, all hematological and serum biochemistry values were different from those of other avians i.e. Psittacines (Polo *et al*., 1998), Capercaillie (Lavin *et al*., 1992), Domestic fowls and Cranes (Hawsky *et al*., 1990), Gulls (Averbeck, 1992), Emu and Ostriches (Eboh *et al*., 1992).

Hematological and blood biochemistry values widely used in avian veterinary medicine (e.g. to permit the interpretation of laboratory

results during disease) for detecting various metabolic and nutritional disorders in these species, but it is important to have hematological data on each species in order to interpret the results accurately for a particular individual. Hematological values are importance for clinical pathological diagnosis such as traumatic injury, parasitism, organic disease, bacterial septicemia and nutritional deficiencies (Ritchie *et al*., 1994). Besides, anemia was defined as the presence of a below-normal red cell count, hemoglobin concentration, and/or packed cell volume (Jain, 1993), and increased mean corpuscular volume (Bush, 1991). Total white blood cell count greater than 10,000 cell/µl was considered suggestive of leukocytosis. The general causes of a leukocytosis include infection, trauma, toxicities, hemorrhage into a body cavity, rapidly growing neoplasms and leukemias. The leukocyte differential aids in the assessment of the leukocytosis. Because a leukocytosis is often caused by inflammation, a heterophilia is usually present. The magnitude of the heterophilia usually indicates the magnitude or severity of the initiating inflammatory process. A leukocytosis and heterophilia can be associated with infectious agents (e.g. bacteria, fungi, chlamydia and parasites). A slight to moderate leukocytosis, heterophilia and lymphopenia can result from either an exogenous or endogenous excess of glucocorticoids (stress response). A lymphocytosis may be expected with antigenic stimulation associated with certain infections. A lymphopenia also may be expected with certain viral disease. A monocytosis can be found with certain disease that produce chemotactic agent for monocytes. These conditions include avian chlamydiosis, mycotic and bacterial granulomas and massive tissue necrosis. An eosinophilia associated with gastrointestinal nematode infections has occasionally been reported. Avian basophils are similar to mammalian basophils in their ability to produce, store and release histamine. Basophils appear to participate in the initial phase of the acute inflammatory response in birds, but this is not always reflected as a basophilia in the leukogram. Increases in plasma glucose levels in blood called diabetes mellitus. Serum glutamic oxaloacetic transaminase is used to evaluate activity of liver or muscle damage while serum glutamic pyruvic transaminases used for evaluation of cell damage. Besides, blood creatinine was derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Severe kidney damage can lead to increase creatinine levels, especially if the filtration rate is decreased (Ritchie *et al*., 1994).

Acknowledgment

We wish to thank to the Khow Kaew Open Zoo for grant, laboratory and facility provided to carry out this research.

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