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Hematological Investigations of Captive Hill Mynah *Gracula religiosa* in Thailand

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Abstract: Hematological values of Hill Mynahs were studied to determine their health or general condition. No statistically significant sex and age differences were found in blood composition except in leukocyte numbers. Red blood cell number ($3.17 - 3.85 \times 10^6 \text{ mm}^{-3}$), hematocrit (46.67 - 48.60 %), hemoglobin content (13.59 - 14.32 g/dl) were not significantly different between adult and young birds in spite of the fact that female birds had smaller amount of these contents than their male counterparts whose values were lower than young birds'. Leukocyte numbers of young birds ($28 \times 10^3 \text{ mm}^{-3}$) and of adult females ($27 \times 10^3 \text{ mm}^{-3}$) were significantly higher than those of adult males ($20 \times 10^3 \text{ mm}^{-3}$). Lymphocytes were the most abundant white blood cells in both adults (48%) and the young (58%). The study provides a database of hematological values of healthy captive Hill Mynahs for diagnosing and monitoring avian medical problems especially in a captive breeding project.

Key words: *Gracula religiosa*, Hill Mynah, red blood cells, white blood cells

Introduction

Hill Mynah *Gracula religiosa*, a talking bird, has a special talent to mimic any sound especially human speech. As a result, people like to have them as caged pets to discharge vocal imitations for human whimsy and the gradual disappearance of wild population for pet market has become a real concern (Archawaranon, 2003). The recent success in Hill Mynah captive breeding will impede the decrement of wild population. Both subspecies (*G.r.intermedia* and *G.r.religiosa*) were bred successfully for three generations at the Zoological Research Station, Ramkhamhaeng University, Bangna Campus, Bangkok, Thailand (Archawaranon, 2002b; 2005).

Hematological screening is conducted in veterinary diagnosis for all captive breeding projects and for the population of vulnerable species (Cooper *et al.*, 1986). Hematological values are useful in determining the health or general condition of birds. Besides, it is also clinically important for diagnosing and monitoring avian medical problems (Leonard, 1982; Cooper *et al.*, 1986). For example, hematocrit and red blood cell counts have generally been used to evaluate the normality of the oxygen transport system (Gessaman *et al.*, 1986) or adaptation (Polo *et al.*, 1992). Besides, among migratory birds, hematocrit has been interpreted in relation to their reproductive condition (Jones, 1983; Keys *et al.*, 1986), circulating hormone levels (Kern *et al.*, 1972), molt status (deGraw *et al.*, 1979), attitudinal shifts (Clemens, 1990), migration (Puerta *et al.*, 1990) and reproductive schedule (Morton, 1994).

This study aimed to provide baseline data on the blood characteristics of healthy captive Hill Mynahs.

Materials and Methods

Blood samples: Blood samples of Hill Mynahs (northern

race) *G. r. intermedia* (Archawaranon, 2002a) were obtained from captive birds at the Zoological Research Station, Ramkhamhaeng University, Bangna Campus, Bangkok, Thailand (Archawaranon, 2002b; 2004a). Birds were kept in six outdoor aviaries, 4 x 8 x 3 m³ in dimension, six to eight birds per aviary, provided with food, water, small houses and perches. Birds were both adult, between 3 - 6 years old, and young, less than one year old and easily identified by pale pink wattles. The gender of Hill Mynahs was specified by sex chromosomes (Archawaranon, 2004b). Adults were composed of 20 males and 15 females. The 10 young were not differentiated by sex. Birds were healthy, with no clinical signs of disease. To reduce diurnal variation in blood parameters (Dolnik, 1973), blood samples were taken almost simultaneously by five people with the same procedure. Blood was drawn with both a syringe containing heparin and heparinized micro capillary tubes from wing vein. Blood smears were also made and fixed at the time of blood collection. The blood samples were analyzed within 24 hrs after collection.

Blood study: Blood was diluted with isotonic saline 1:62,500 for red blood cell counts. Red blood cells (RBC) were counted with Cell-dyn 300 hematology analyzer operated by electronic resistance principle. Next hematocrit was determined by centrifuging a micro hematocrit tube of blood for five mins at 11,000 rpm. Hematocrit or packed cell volume was read with a micro hematocrit reader. As for hemoglobin concentration, it was measured by diluting blood with isotonic saline 1:250 and then adding the well-mixed blood to potassium cyanide, potassium ferricyanide and acetic acid solution to let out hemoglobin from RBC. This sample was centrifuged at 2,500 rpm for 10 mins. The supernatant was measured to obtain hemoglobin value

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Table 1: Hematological values of the Hill Mynahs (mean ± SD)

	Adult males (n = 20)	Adult females (n = 15)	Young (n = 10)
Erythrocytes (10 ⁶ cells mm ⁻³)	3.75 ± 0.44	3.17 ± 0.43	3.85 ± 0.59
Hematocrit (%)	47.60 ± 4.86	46.67 ± 3.64	48.60 ± 2.46
Hemoglobin (g/dl)	14.26 ± 1.18	13.59 ± 1.12	14.32 ± 0.62
MCV (μm ³)	126.04 ± 11.69	124.23 ± 8.68	131.83 ± 13.89
MCH (pg/cell)	38.35 ± 3.57	37.69 ± 2.62	38.86 ± 4.47
MCHC (g/dl)	30.06 ± 1.53	29.08 ± 0.99	29.46 ± 0.52
Leukocytes (10 ³ cells mm ⁻³)	20.77 ± 5.82	27.04 ± 6.80	27.88 ± 7.38
Heterophils (%)	43.79 ± 8.02	43.43 ± 7.91	34.75 ± 6.33
Lymphocytes (%)	48.65 ± 7.54	48.25 ± 8.47	58.44 ± 12.39
Eosinophils (%)	4.08 ± 2.47	3.75 ± 3.64	3.11 ± 0.63
Basophils (%)	0.80 ± 0.73	0.27 ± 0.38	0.20 ± 0.40
Monocytes (%)	4.60 ± 4.08	4.30 ± 3.19	4.30 ± 3.19

by automatic hematology analyzer. The hematimetric indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the RBC count, hematocrit and hemoglobin concentration.

Leukocytes or white blood cell counts were done twice. Blood was diluted with isotonic saline 1:250 and then the well-mixed blood was added to potassium cyanide, potassium ferricyanide and acetic acid solution to break RBC. Leukocytes were first counted with hematology analyzer and then counted again from blood smears. Blood smears were stained with Wright's stain. Identifying and counting leukocytes was done with a light microscope with oil immersion lens (X100). At least 150 white cells were counted in each sample. It was found that heterophils and lymphocytes were the most common leukocytes. Heterophils were round cells with a colorless cytoplasm and eosinophilic rod-shaped granules together with a two lobed nucleus. Lymphocytes were typically round cells with a big nucleus almost full of the cytoplasm with densely clumped chromatin and pale blue cytoplasm. Eosinophils were normally round with cytoplasm stained clear, pale blue in contrast to the colorless cytoplasm of heterophils and contained round eosinophilic granules. Eosinophil nuclei were lobed with coarse and clumped chromatin that stained purple and bluer than heterophil nuclei. Basophils were round cells with round, centrally located nuclei. The nucleus stained light blue and was often hidden by the cytoplasmic granules which were deeply basophilic. Monocytes were large leukocytes having irregular shapes. The nuclei varied in figure from round to bilobed. The cytoplasm stained blue-gray and contained two distinct parts: lightly- and deeply-stained areas.

Data on blood were compared for any difference between sex and age by using Student's t-test.

Results

Red blood cells: A comparative analysis of red blood

cells showed no statistically significant sex and age differences. No differences were found in the numbers of erythrocytes, hematocrit or packed cell volume, hemoglobin content and hematimetric indices (Table 1). However, all parameters relating to red blood cells were smaller in adult females than in adult males and the young. Meanwhile these numbers of the young were higher than those of adult males though not significantly different.

White blood cells: The mean leukocyte count of the young was significantly higher than of adult males (t = -2.54, p< 0.05), it was not significantly different from that of adult females. The leukocyte number of adult males was significantly smaller than that of adult females (t = -3.74, p< 0.05). Lymphocytes were the most abundant white cells in both adults (48%) and young (58%). However, heterophil counts in the young averaged lower than in adults (t = 3.23, p< 0.05) but lymphocyte counts in the former were higher (t = -3.07, p< 0.05). Adult male basophils were significantly higher than adult females (t = 2.51, p< 0.05) and young (t = 2.38, p< 0.05). No age- or sex-related differences were found in the number of eosinophils and monocytes (Table 1).

Discussion

Hematological values of Hill Mynahs from this study tended to be higher for male birds than female ones. Sex, age, diet and hormone are known to influence blood cell absolute and relative numbers (Puerta *et al.*, 1990). In general, the amount of RBC, hematocrit and hemoglobin level are higher in male than female birds (Campbell and Dein, 1984; Sturkie, 1986). The increase of red cell numbers in male quails due to androgen and the reverse effect of estrogen (Nirmalan and Robinson, 1972). However, with continued estrogen administration in chickens, erythropoiesis is not depressed (Gilbert, 1963). Although my data showed no statistically significant differences in RBC, hematocrit, hemoglobin or hematimetric indices with reference to sex, they were

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consistent with this trend.

It is proposed that environment (Puerta *et al.*, 1990) or seasonal change (Morton, 1994) can also affect hematological values. Chickens raised at the temperature of 10°, 21.1° and 32°C had higher hematocrits and hemoglobins than those raised at lower temperature regardless of dietary regime (Kubena *et al.*, 1971). Migratory birds revealed seasonal variation in several blood components which also occurred in captive birds (Kern *et al.*, 1972; Chilgren and deGraw, 1977; deGraw *et al.*, 1979). In migratory birds, flight increased the oxygen demand (Lasiewski, 1972; Berstien *et al.*, 1973) and it followed that hematocrit, hemoglobin content and red cell numbers were higher in good fliers than in flightless species (Viscor *et al.*, 1985). However, the study on hematology of wintering common cranes found that young and adult cranes had similar erythrocyte numbers, blood hemoglobin levels and hematocrit (Puerta *et al.*, 1990). As Hill Mynahs, young and adult birds had similar RBC numbers, blood hemoglobin levels and hematocrits. Hematological values in both captive and free-living birds were recorded for many bird species and there were reports on the similarity of blood composition between captive populations and free-living ones (deGraw *et al.*, 1979; Puerta *et al.*, 1990). Nevertheless, a study on blood composition in free-living Hill Mynah should be carried on in order to compare the results between adult and young wild birds.

Previous works reported that leukocyte numbers of birds ranged from 15 to 30 ($\times 10^3 \text{ mm}^{-3}$) (Sturkie, 1986). Hill Mynah values in this study were in this range. The level of leukocytes in adult female Hill Mynahs averaged higher than in adult males. Similar results were found in chickens (Lucas and Jamroz, 1961) and quails (Nirmalan and Robinson, 1972). It was put forth that estrogen administration increased leukocyte counts in male quails (Nirmalan and Robinson, 1972) and in chickens (Meyer, 1973). The young had more leukocytes than the adults. It is possible a probably higher risk of infection in the captive young gives them a higher number of leukocytes (Puerta *et al.*, 1990). Differential leukocyte counts from this study were not different from the range reported for several species (Sturkie, 1986). Lymphocytes were the most abundant white cells. Some authors described the heterophils as the more numerous leukocytes in certain species and not in others (Leonard, 1982; Cooper *et al.*, 1986). These numbers vary so much that it may be due to different methodologies (Sturkie, 1986).

This study gives a database of hematological values of healthy captive Hill Mynahs which are primarily important to clinical pathology diagnosis for a captive breeding project. Most blood values determined in my study were within the range of variations reported for different bird species (Gee *et al.*, 1981; Leonard, 1982; Sturkie, 1986).

A further study on seasonal changes of blood values in both free-living and captive Hill Mynahs is suggested to add a perspective of avian hematology.

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