DIFFERENTIAL FEATURES OF ERYTHROCYTES OF AFRICAN FREE RANGIG CHICKEN (*GALLUS GALLUS DOMESTICUS*) AND THE GREATER FLAMINGO (*PHOENICOPTERUS RUBER ROSEUS*) OF LAKE MANYARA NATIONAL PARK

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SUMMARY

Erythrocytes of the Greater flamingo, *Phoenicopterus ruber roseous*, and those of the free ranging chicken, *Gallus gallus domesticus*, differed characteristically in sizes, cytoplasmic and nuclear staining, shapes and nuclear shapes. Erythrocytes of the greater flamingo were spindle shaped and more elongated and significantly larger than those of the free ranging chicken. The cytoplasm is scanty and stains more eosinophilic (acidic) compared to erythrocytes of the free ranging chicken. The nuclei in flamingo's erythrocytes are distinctive, large, oval, elongated to spindle shaped and centrally located. The nuclei are banded or striated across the length. Majority of chicken erythrocytes are oval to round, with an oval nuclei. The cytoplasm is less eosinophilic compared to erythrocytes of the greater flamingo. Respective means ± standard deviations of erythrocyte counts (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in the free ranging chicken and Greater flamingo were; RBC: 2.0±0.6 and 2.5±0.6 $x10^{12}$/l, Hb: 13.3±3.6 and 9.5±1.0 mmol/l, PCV: 0.32±0.065 and 0.47±0.05 1/l, MCV: 176.3±50.6 and 174.4±42.4 fl, MCH: 66.3±27.4 and 39.3±6.0 pmol and MCHC values of 39.0±9.50 and 20.5±4.5 mmol.

INTRODUCTION

The African free ranging chicken is the commonest protein source to the rural and urban poor human communities (Minga *et al.*, 1989; Cooper, 1995), because New Castle disease, infectious bursitis, pullorum disease, fowl typhoid, cholera, fowl pox, avian encephalitis, coccidiosis, Marek's disease and avian leukosis complex, and the lack of feeds have severely limited the exotic commercial bird industry. Nutritional deficiencies have also

reduced flock production capabilities and there are no reliable markets for eggs and chickens because of the poverty in the people. The free ranging chickens though very susceptible to Newcastle disease tolerates other diseases and feeds by scavenging only.

The demand for feeding the increasing human population necessitates the rearing and utilization of domestic birds and traditionally non-food wild birds, reptiles, fish and crustaceans.

Necropsy examination confirms diseases causing mortalities in individual birds (Mellau and Cooper 1994), but use of blood analysis in diagnosis of diseases is advantageous in live birds in that it enables recognition of general health in individuals and flocks and the environment (Zinkl, 1986). Very small blood samples adequately allow diagnosis in ill and wounded birds and genetic identification (Minga and Nkini, 1993; Samour *et al.*, 1994a; 1994b). Blood analysis is important in determining the causes of morbidities and mortalities, health monitoring, prevention and control of diseases in poultry and wild birds. Blood analysis enables detection of nutritional disorders and parasitic diseases in commercial poultry, guinea fowls, turkey, ducks and pigeons (Hodges, 1977; Awotwi and Boohene, 1992, Cooper, 1995; Cooper *et al.*, 1996).

However the haematological information, as a basis of diagnosis of diseases is very scanty or lacking in many African birds.

In wild birds haematological data is reported on the burn owl, spotted eagle, African harrier, hooded vulture, lizard buzzard, lanner falcon and towny eagle (Cooper, 1972; 1975). A number of investigations have also been done in flamingos (Hawkey *et al.*, 1984a; b). Blood values are available for Rosy (Caribbean) flamingos (*Phoenicopterus ruber ruber*), greater flamingo (*P. ruber roseus*), Andean flamingo (*P. andenus*) (Bush and Smith, 1980; Seidil 1980), red tailed hawks (Rehder *et al.*, 1982), kestrel (Kirkwood, 1979) and bustards (Samour *et al.*, 1994a; Howlett *et al.*, 1995; D'Aloia *et al.*, 1996). Because of availability of information on bustards and falcons their captive breeding have been very successful, particularly in Arabia and Persian Gulf countries (Samour *et al.*, 1994a).

Rearing of any animal requires knowledge on specific diseases that affect them, their diagnostic methods, treatment and prevention. The increase in captive rearing of flamingos, ostriches, francolin, Guinea-fowls and pigeons for economic, ornament and tourism, and captive breeding of endangered species such as crowned crested cranes requires comprehensive knowledge on avian haematology, the first aid to diagnosis of diseases and to recognition of haemoparasites and malignancies (Cooper, 1995;

Samour *et al.*, 1994a). Although avian haematology has played a pivotal role in defining immunologic system for viral etiology of blood neoplasia (Hodges, 1977), the immunological responses of avian blood cells to diseases are not fully understood. Considerable variations in blood cell morphology also exist in animals (Hawkey and Dennett, 1989) and it is possible to identify animal species by examination of blood cells on smears, but the markers for species identification on blood films have not been defined. This work was to study the differential features of erythrocytes of particularly the African free ranging chicken and the greater flamingo for the purpose of identifying them on the blood smear for forensic purposes. Since it has not been so far possible to identify species of birds on blood smears this information shall be useful in conservation and bird traces in trade.

MATERIALS AND METHODS

Blood samples were collected from the brachial vein in vacuum tubes (Vacutainer^R, Becton-Dickinson England) containing potassium ethylene diaminotetraacetate (K_3EDTA), from 146 African free ranging chickens (*Gallus gallus domesticus*) and 9 greater flamingo (*Phoenicopterus ruber roseus*). The chickens were obtained from the open city market in Morogoro, Tanzania, surrounding villages and on sale by the road. Blood samples from the Greater fiamingo were collected following capture at Lake Manyara in the Rift Valley. The chickens and flamingos were physically examined to detect any pathophysiological conditions, including presence of external parasites, visible body lesions, clinical signs of disease and nutritional adequacy and only apparently healthy birds free from disease signs, bright and alert were used for this study.

Haemoglobin concentration was determined by cyanmethemoglobin method. Packed cell volume (haematocrit) was determined in Sigma 201 M microhaematocrit centrifuge at 12000 g (Sigma Co., USA). Erythrocyte counts were determined by haemocytometer technique. Mean corpuscular volumes (MCV, (x 10^{-15})), mean corpuscular haemoglobin (MCH, picomol (x10^{-13})) and mean corpuscular haemoglobin concentration (MCHC mmol) were calculated from RBC, PCV and Hb values. Blood smears were stained with Giemsa stain for examination of blood cell morphology. Data were analyzed by the general linear models procedure (SAS, North Carolina, 1985).

RESULTS

There were significant differences between species in the values of blood parameters, particularly between large and small breeds. The ranges for RBC counts in the chicken and greater flamingo were 0.86 - 3.5 and 2.6 - 3.6 $x10^{12}/l$ respectively, and together with the mean ± standard deviations of Hb, PCV, MCV, MCH and MCHC are indicated in Table 1.

Table 1: The ranges and mean ± standard deviation of RBC counts (x $10^{12}/l$), Hb concentration (mmol/l), PCV (l/l), MCV (fl), MCH (pmol) and MCHC (mmol/l) in the chickens and flamingos.

Bird	RBC	Hb	PCV	MCV	MCH	MCHC
Chicken	0.9-3.5 2.0±0.64	5.8-19.4 13.3±3.6	0.23-0.52 0.32±0.06	105.7-183.2 176.3±50.6	29.1-83.6 66.3±27.4	21.0-66.0 39.0±9.5
Flamingo	2.6-3.6 2.5±0.60	8.0-11.8 9.5±1.00	0.35-0.58 0.47±0.05	125.8-305.6 174.4±42.0	26.1-53.9 39.6±6.0	19.6-28.0 20.5±4.5

In blood films stained with Giemsa stain presented in Figures 1-4, greater flamingo erythrocytes were distinctively oval, elliptical or spindle shaped with oval or spindle shaped nuclei surrounded by evenly stained eosinophilic cytoplasm. Immature cells were also seen, smaller than mature ones, round to ovoid in shape with round nuclei and polychromatic cytoplasm. The erythrocyte shapes and staining affinities varied between species and were very characteristic for flamingos and free ranging chickens that they can be used to identify the species on a blood smear (Fig. 1).

In the flamingo erythrocytes measured 12.1-15.3 µm in the long axis (13.5±0.9 µm) and 5.8-7.9 µm (7.0±0.5) wide. Nuclear lengths and widths were 5.3–7.4 µm (6.0±0.5) µm and 2.1–3.2 µm (2.5±0.3) respectively. The ratio of the nucleus to whole cell along the length and width respectively were 0.4–0.6 and 0.3–0.5 respectively. In the free ranging chicken erythrocytes measured 9.5-14.2 µm (11.1±1.0) in the long axis and 4.7-9.5 µm (7.0 ±0.6 µm) in width. Nuclei were 3.7-6.3 µm (4.8±0.6) long and 2.1-3.7 µm (3.1±0.4) wide.

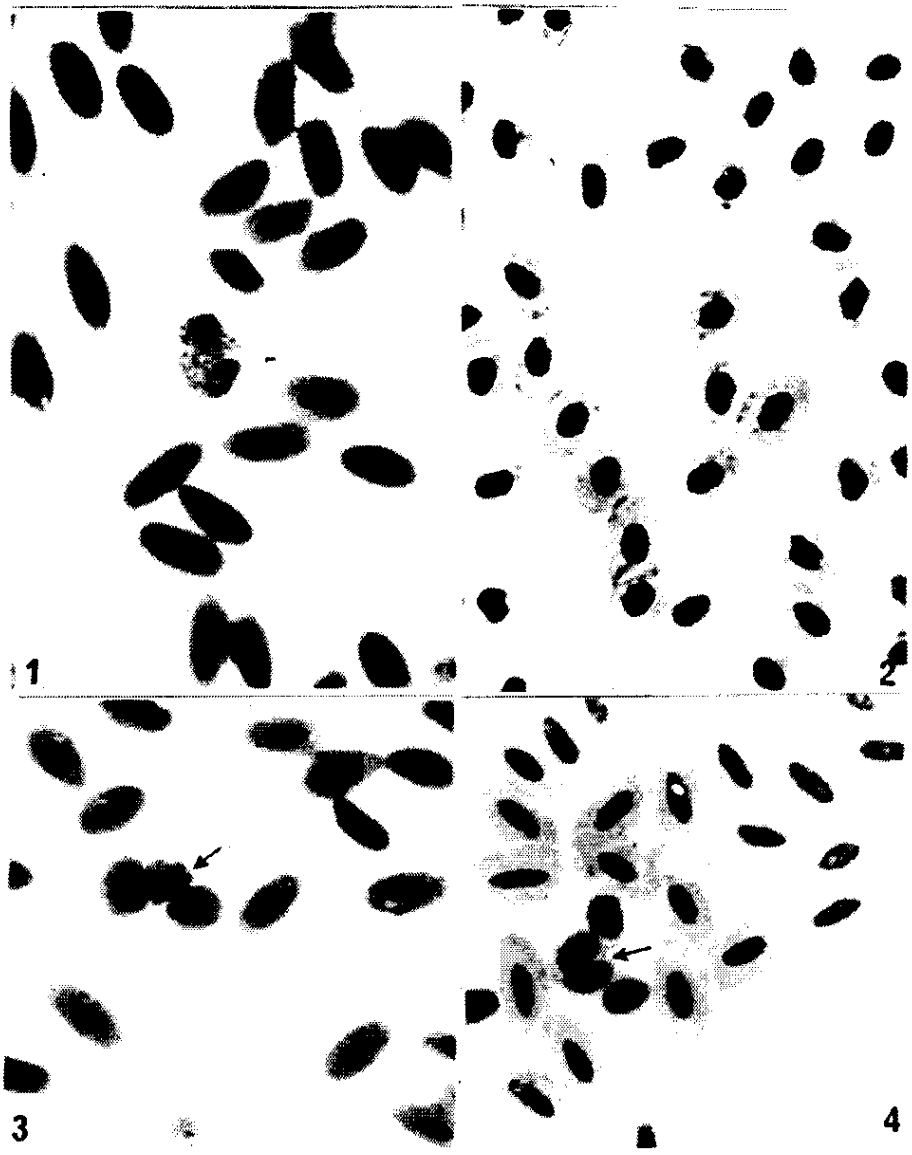

Figure 1: Blood smear of the greater flamingo, *Phoenicopterus ruber roseus*, showing large spindle shaped red blood cells Giemsa stain x 1897.5.

Figure 2: Blood smear of the free ranging chicken, *Gallus gallus domesticus*, showing more round erythrocytes. Giemsa stain x1897.5.

Figure 3: Blood smear from flamingo showing round to oval thrombocytes (arrow). Giemsa stain x1897.5.

Fig. 4: Blood smear from a greater flamingo showing large ovoid to round thrombocytes (arrow). Giemsa stain x1897.5

The nuclear to whole cell ratio along length and width respectively were 0.3–0.6 and 0.3-0.7. The cytoplasm in flamingo's erythrocytes was dark pinkish, while the nucleus had some bands or striations across length.

Erythrocytes of the free ranging chicken were therefore significantly smaller than those of the greater flamingo ($p<0.05$),

more ovoid to round, more compact, pale staining cytoplasm containing clear homogenous structure (Fig. 2). Those of the greater flamingo were larger than in the chicken, more spindle shaped to elongated, containing more course cytoplasm with evenly stained and more oesinophilic heterogeneous appearance of organelles. On the basis of these characteristics it is possible to identify the flamingo from other species of birds by examination of the blood smears.

Thrombocytes were smaller than the erythrocytes in both the chicken and the flamingo, but oval in shape with rounded and blunter ends than those of red cells, being round, irregular or elongated (Figs. 3-4). The cytoplasm varied in amount, frequently non-homogenous and having reticular appearance, while the nuclei were round. Thrombocytes of chicken measured 5.1±0.37 μm in diameter, while nuclei were 2.8-4.6 μm (mean 3.4±0.3) in diameter. Flamingo's thrombocytes measured 6.2±2.5 μm in diameter, while their nuclear sizes were 2.1-5.3 μm in diameter (4.5±1.2 μm in diameter). Thrombocyte of the free ranging chicken were small, round, non-homgenous, staining with granular densely eosinophilic structures, while those of the grater flamingo were large, oval, with variable cytoplasmic amount staining pale blue.

The salient differentiating morphological features between the erythrocytes of the flamingo and chicken are sizes, shapes, staining characteristics of cytoplasm, nucleus and granules, shapes of nuclei and distribution of nuclear chromatin. Erythrocytes of the greater flamingo are larger than those of the free ranging chicken, spindle shaped and more elongated. The cytoplasm is scanty, more acidic than in the chicken erythrocytes and contains oval to spindle shaped nuclei. The nuclei are large, elongated and centrally located. Majority of erythrocytes of the free ranging chicken are oval to round and the nucleus is round or oval. The cytoplasm is less eosinophilic than that of greater flamingo's erythrocytes. Some chicken erythrocytes have a narrow rim of more pale staining cytoplasm around the nucleus. In the flamingo the erythrocyte nucleus appears banded or striated across the length because of the scattering of euchromatin. On the basis of these characteristics the flamingo can be identified unequivocally by examination of erythrocytes on Giemsa stained blood films.

DISCUSSION

The data observed in this study on erythrocytes of the free ranging chicken and flamingo are comparable to those reported for chickens (Archer and Jeffcott, 1977; Zinkl, 1986). Variations in blood chemistry in aves are very great, being influenced by feeds,

species, breed, age, sex and different degrees of infections by helminthes and virus (Awotwi and Booheene, 1992).

RBC counts are found to differ between male and female chickens, geese and quails because hormones like erythropoietin, androgens, corticosteroids, somatostatin and thyroxine (Nirmalan and Robinson, 1971; How *et al.*, 1979). RBC and PCV levels of guinea fowls are lower than those of ducks and turkeys (Awotwi and Boohene, 1992; Cooper *et al.*, 1996). RBC, Hb and PCV are lowest while MCV is highest in neonates.and juveniles (Hawkey *et al.*, 1993; Mbassa and Poulsen, 1991a; b; c, D'Aloia et al., 1995).

Chickens had higher Hb concentration compared to the flamingo at 5.8-19.4 mmol/l, considerably higher than those of Zinkl (1986) for exotic chickens (4.0-8.1 mmo/l). Hb concentration increases in cold weather (Deaton *et al*, 1969), due to increase RBC, MCV and PCV (Hodges, 1977).

In this study RBC counts for free ranging chickens were 0.86 - 3.5 $x10^{12}/l$ comparable to 2.0-3.5 x $10^{12}/l$ (Zinkl, 1986) but lower than 3.0-4.5 x $10^{12}/l$ of Soliman *et al.* (1966). These counts are within ranges of houbara bustard values of 2.5 $x10^{12}/l$ (Samour *et al.*, 1994b). Free ranging African chickens appear to have very variable levels, probably because these birds are freely ranging, on very poor nutrition and exposed to different disease agents, internal and external parasites.

PCV in birds of the present study (0.23.0 –0.58) at 26-28°C, are within 0.22.0-0.35 for chickens (Zinkl (1986).

MCV values for chickens were 105-384 fl, higher than those of Zinkl (1986) at 90-140 fl, but comparable to those of bustards at 152 fl (Samour *et al.*, 1994b).

MCH values were comparable to those of Zinkl (1986) at 33.0-47.0 pmol and 30.2 pg for 8 week old birds of Soliman *et al.* (1966).

MCHC in birds of this study ranged from 19.6 to 39.0 mmol. Other studies reported MCHC for chickens, ducks and turkeys to be 30-40 g/dl (Jones and Johansen 1972). Soliman *et al.* (1966) obtained 23.9 g/dl for 8 weeks chickens, while Abou-Ashour and Edwards (1972) reported 29.5 g/dl for mature hens. Samour *et al.* (1994b) reported 31.9 g/dl for bustards.

The variations in levels of avian blood parameters are very great and are due to many factors, feeds, age and sex. The difficulties in aging birds are particularly major sources of the reported disparities in data comparisons.

The sizes of erythrocytes also greatly vary, mainly because of species characteristics and adaptations (Table 2).

Table 2: Reported blood cell sizes (μm) in length and width of avian blood cells

Cell type	Chicken		Flamingo	
	Length	Width	Length	Width
RBC	11.1[1]	7.0[1]	13.5[1]	7.0[1]
	10.7-13.0[2]	6.5-7.2[2]		
Thrombocyte	6.1[1]	4.8[1]	6.2[1]	3.9[1]
	8.1 – 10[2,3]	3.9 – 5.0[2,3]		

[1]Present study, [2]Freeman, 1971, [3]Lucas and Jamroz 1961.From the results of this study, the differences between the blood smear of a greater flamingo and a free ranging chicken are clear and any forensic identification can be achieved. It is evident in (Fig. 1, 2) that the erythrocytes of the free ranging chicken differs very much from the Flamingo's erythrocytes. The free ranging chicken has more of ovoid to roundish shape RBC, while flamingo has spindle shaped. The shape of the nucleus follows that of the cell. Chicken erythrocytes are smaller in size than those of the flamingos (Table 1; Fig. 1), but no significant differences were observed on the cell widths. Thrombocytes are round in the free ranging chicken, but oval cells in flamingo (Fig. 3).

It can be concluded unequivocally that flamingos and chickens can be distinguished from the morphology of erythrocytes on a blood smear and this is a tool for forensic identification of species irrespective of age.

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