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The Comparative Study of the Blood Cellular Composition in Muscovy Ducks in Nigeria

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Abstract: Twelve blood samples were obtained from four male and eight female adult Muscovy ducks which were raised semi-intensively. The total and differential blood counts as well as study of the blood cellular characteristics were carried out in this study. We report herein that the male Muscovy ducks had a higher average erythrocyte count ($2.5 \times 10^6/\text{mm}^3$) than the female Muscovy ducks ($2.20 \times 10^6/\text{mm}^3$). The Muscovy ducks average erythrocyte counts were lower than the average counts reported for temperate breed of ducks but higher than those reported for Nigerian domestic chicken. No significant differences in the average total leucocyte count between the male and female Muscovy ducks was observed. The average total leucocytes count for the Muscovy ducks were higher than those reported for the temperate breeds of ducks and the Nigerian domestic chicken. Higher average lymphocyte count was recorded for the male Muscovy ducks (72.75%) than the female Muscovy ducks (61.37%) while a lower basophil average counts was recorded for the male Muscovy ducks (0.75%) than the female Muscovy ducks (1.5%). The eosinophils had a diverse number of lobulation on their nucleus and spherical intracytoplasmic granules while the heterophils had an irregular cellular outline and a spindle shaped intracytoplasmic granules. Taken together, to the best of our knowledge, this is the first comparative study of the blood cellular composition in Muscovy ducks in Nigeria

Key words: Muscovy ducks, blood counts, comparative, male, female

INTRODUCTION

Ducks belong to the family Anatidae of the order Anseriformes and are closely related to geese. Wild ducks have long been favourite game birds and are hunted for sports, food and feathers www.wikipedia.com (Wikipedia accessed 24/03/10).

Ducks have been domesticated for many centuries and are raised commercially for their meat and eggs, depending on the kind of ducks raised. Duck meat is dark, rich in iron and B-vitamins and is fattier than chicken or turkey meat www.wikipedia.com (Wikipedia accessed 24/03/10).

Ducks ranked second in population in Africa amongst domestic birds particularly in tropical Africa, with an estimated population of 5,189,000 after fowls with an estimated population of 410,346,000. Most world population of ducks are however found in Asia and Europe (Nesheim *et al.*, 1979).

There are different species and breeds of ducks found in the world depending on the locality they exist. In Africa, two different breeds of ducks, together with cross between them are found. This includes but not limited to the Muscovy or Barbary Ducks. These are the common breeds of ducks in Nigeria. They have a black and white plumage and are recognized by a fleshy red protuberance around the eyes and beak. They are mainly raised for meat and are believed to have been imported from the United States (Nesheim *et al.*, 1979).

Detailed information on the cellular description of the blood of ducks is not readily available thus we focus our review of literature mainly on that of the domestic fowl, with occasional reference to the available information on studies carried out on blood of other breeds of ducks (Roger and Carlton, 1967).

According to Bell and Freeman (1971) the blood of the domestic fowl is divided into the cellular part and the fluid part. The cellular component is composed of three (3) major types of cells:

- (a) Erythrocytes
- (b) Leukocytes
- (c) Thrombocytes

The leukocytes are further divided into:

- (i) Granular leucocytes (Heterophils, eosinophils and basophils).
- (ii) Non-granular leucocytes (Lymphocytes and monocytes).

The fluid part is the blood plasma and its constituents. The blood cells are suspended in the plasma. In an earlier study conducted by Roger and Carlton (1967) they observed that there was a significant differences in the number and size of the blood cells studied in different breeds and species of ducks with age, sex, breed, body weight and season.

Granular leucocytes

Heterophils: In man and other animals, these leucocytes were noted to process neutral staining granules thus were called neutrophils. However, in birds and rabbits, they are acidic in reaction when stained (Olson, 1937). The characteristic features of the heterophils were the presence of spindle or rod shaped acidophilic crystalline bodies in the cytoplasm. In routinely stained smears, the cytoplasmic bodies were distorted and then be variable in shape. The bodies were reported to be a distinct and sometimes brilliant red against a background of colourless cytoplasm. The nucleus was observed to be polymorphic with varying degrees of lobulation (Olson, 1937).

Numbers of heterophils reported in the literature is from 3,000 to 12,000 per mm³ which makes the cell type the second most common leucocytes in the blood of the fowl (Bell and Freeman, 1971).

Esinophils: Esinophils of the domestic fowl vary more in size than heterophils. The cytoplasm has a faint yet distinct-gray tint (Bell and Freeman, 1971). The granules were spherical and relatively large with a dull red colour, compared to the brilliant red of heterophils granules (Bell and Freeman, 1971). The esinophils and heterophils were said to be difficult to distinguish from each other in avian blood (Olson, 1937). Esinophils counts for about 2% of the total leukocytes in the domestic fowl's blood (Bell and Freeman, 1971).

Basophils: Basophils have been previously reported in the domestic fowl to be slightly smaller than heterophils and were noted to have strongly basophilic granules (Bell and Freeman, 1971). The nucleus was also masked by the granules. The nucleus was round or oval in shape and may at times be lobulated. The cytoplasm was abundant and devoid of colour. The nucleus was however weakly basophilic. The number of basophils and esinophils in the blood of normal domestic fowl appears to be much the same (Bell and Freeman, 1971; Olson, 1937).

A review of past literature revealed that differential counts were one of the most controversial values in ducks. The cause of controversy was centred on the difficulty in distinguishing esinophils from heterophils.

Magath and Giggins (1934), stated that lymphocytes comprise the greatest number of leucocytes ranging from 45.5-83.0% with a mean of 61.7%, monocytes number ranged from 4.0-20.0%, Basophils ranged from 0.0-4.0%.

Hewitt (1942) made no attempt to distinguish between esinophils and heterophils and stated that important staining variations can cause confusion in polymorphonuclear cells. He then classified the polymorphs into heterophils with ellipsoidal rods and heterophils with bacillary rods to avoid the problem of distinguishing the heterophils from the esinophils.

Didisheim *et al.* (1959) reported a count of 60% lymphocytes, 11% monocytes, 4% basophils and 25% esinophils. Apparently, the problem of distinguishing between heterophils and esinophils was avoided by listing all polymorphonuclear cells, other than basophils as esinophils.

An interesting theory proposed by Lucas and Jamroz (1961) was considered in evaluating data on total white blood cells. According to these investigators, the specific granules of esinophils have two morphologic forms with transitional stages from granular to rod forms both in an individual bird and within species. These authors reported their average counts on ten adult mallard ducks with no listing of sex of birds as: - Lymphocytes - 32%, monocytes - 8%, heterophils - 48%, esinophils - 7% and basophils - 5%.

Thus this study was conducted in order to achieve the following objectives:

- To obtain blood cellular value and note the cellular characteristics of some blood cells of the Muscovy ducks.
- Note any differences in the blood cellular picture of the Muscovy duck with those reported for exotic (temperate) breeds of ducks and the domestic fowl.
- Note any differences in the blood values of the male and female Muscovy duck.

MATERIALS AND METHODS

Twelve (12) healthy Muscovy ducks (8 females, 4 males) were utilized for this study. Blood samples were obtained from the 12 healthy Muscovy ducks by venipuncture via the cephalic vein into sample bottles containing Ethylene Diaminetetraacetic Acid (EDTA) as anticoagulant. The ducks were dewormed a week prior to sampling using a commercially prepared agent, piperazine.^(R) The ducks were raised semi-intensively.

Stain preparation: 250 mg of May-Grunwald stain powder was dissolved in 100 ml of absolute methyl alcohol by heating in a flask plugged with cotton-wool in a water bath. The solution was filtered after cooling. Giemsa stain was prepared in exactly the same manner as the May-Grunwald stain.

Staining procedure: Thin blood smear was prepared from the blood sample and air dried. 1 ml of May-Grunwald solution was added to the slide for 3 min. The same volume of distilled water was then added and the slide was then added and the slide was rocked to mix. The film developed a pinkish tinge after a minute or two and the stain was drained off, without washing. The slide was then flooded with giemsa solution which had been diluted a further 1:10 (drop of stain in 10 ml of distilled water). After 15 min, the stain was drained off and the slide was wash with distilled water for 1 min after which it was dried and examined under the microscope.

Leucocyte count: 3.80 g sodium citrate, 0.05 g Brilliant cresyl blue and 0.22 ml of Neutral formaldehyde were dissolved in distilled water to a volume of 100 ml. The diluents were filtered before use.

The blood sample was well shaken to mix and a 1:200 dilution was prepared using a Red cell diluting pipette to aspirate blood to the 0.5 mark. The diluents were then aspirated to the 101 mark. The pipette was shaken for 4 min. The first few drops from the pipette were discarded. Both sides of the haemocytometer were filled with the diluted blood and allowed to settle for 15 min. The leucocytes were then enumerated and the total leucocyte count calculated by:

Averaging the counts obtained from the two chambers

$$\text{Leucocytes/mm}^3 = \frac{\text{Cells counted} \times \text{Dilution factor}}{\text{Volume counted in mm}^3}$$

Erythrocyte count: The blood sample was well shaken and a 1:200 dilution was prepared by aspirating the blood to the 0.5 mark and then aspirating the diluents (0.85% saline solution) to the 101 mark of the red cell diluting pipette. The pipette was shaken for few minutes before filling the haemocytometer. The cells were allowed to settle for a few minutes.

Under high powered magnification, the cells were counted. Total erythrocyte count obtained was calculated by:

Averaging the counts obtained from the two chambers.

$$\text{Leucocytes/mm}^3 = \frac{\text{Cells counted} \times \text{Dilution factor}}{\text{Volume counted in mm}^3}$$

RESULTS

The average erythrocyte count obtained showed that the male Muscovy ducks have a higher average erythrocyte count $2.52 \times 10^6/\text{mm}^3$ than the female Muscovy ducks $2.20 \times 10^6/\text{mm}^3$ (Table 1). The erythrocytes were elliptical and nucleated, with orange-blue or greyish ink cytoplasm (Fig. 1, lightning bolt arrow).

The average total leucocyte count obtained for the Muscovy ducks indicates that there was no significant difference in the total leucocyte count between the male and female Muscovy ducks (Table 1).

The differential cell count showed that the lymphocytes were the majority of leucocytes. The male Muscovy ducks had a higher average lymphocyte count 72.75% than the female Muscovy ducks 61.37% (Table 2). The lymphocytes showed variability in size and had a thin cytoplasm bordering the nucleus (Fig. 1 arrow down and Fig. 2).

Table 1: Total erythrocytes and leucocytes counts for male and female muscovy ducks

Sample number	Sex of duck	Total erythrocytes count $\times 10^6/\text{m}^3$	Total leucocyte count $\times 10^6/\text{m}^3$
1	Female	2.10	30.20
2	Female	2.31	3060.00
3	Female	2.22	26.50
4	Female	2.20	35.00
5	Male	2.41	31.00
6	Male	2.60	30.60
7	Male	2.48	34.21
8	Male	2.59	28.70
9	Female	2.30	33.20
10	Female	2.11	32.10
11	Female	2.00	28.51
12	Female	2.41	31.33
Mean	Female	2.20	30.93
	Male	2.52	31.13
	Total	2.31	30.99



Fig. 1: Photomicrograph showing various blood cells of Muscovy ducks; Erythrocyte (lightning bolt arrow), Lymphocyte (down arrow), Heterophil (right arrow) and Eosinophil (data store arrow) $\times 100$

There was no significant difference in the average heterophils count, between the male and female Muscovy ducks (Table 2). Additionally the heterophils had an irregular cellular outline and the nucleus was polymorphous. The cytoplasmic granules were rod shaped and acidophilic (Fig. 1, right arrow). The eosinophils also showed similar characteristic with the heterophils but the eosinophils had a more regular cellular outline and oval or round intracytoplasmic granules (Fig. 1, data store arrow).

The basophils in the male Muscovy ducks had a lower average count 0.75% than the female Muscovy ducks 1.5%. The basophils were generally the less common leucocytes in the blood constituents of the Muscovy ducks (Table 2). The basophils were ovoid in shape, with a slight basophilic to colourless cytoplasm, with strongly basophilic intracytoplasmic granules (Fig. 3, lightning bolt arrow).

Table 2: Differential leucocytes counts for male and female Muscovy ducks

Sample number	Sex of duck	Lymphocyte count (%)	Heterophils count (%)	Monocytes count (%)	Esinophils count (%)	Basophils count (%)
1	Female	64	23	8	4	1
2	Female	62	14	7	4	2
3	Female	57	25	12	5	2
4	Female	60	20	10	3	2
5	Male	68	16	8	2	2
6	Male	81	21	8	4	0
7	Male	72	13	7	4	1
8	Male	70	19	10	6	0
9	Female	66	21	12	3	2
10	Female	59	22	8	3	1
11	Female	62	15	10	4	1
12	Female	61	21	5	6	1
Mean	Female	61	20.50	9.00	4.00	1.5
	Male	72.75	17.25	8.25	4.00	0.75
	Total	65.16	19.416	8.75	4.00	1.25

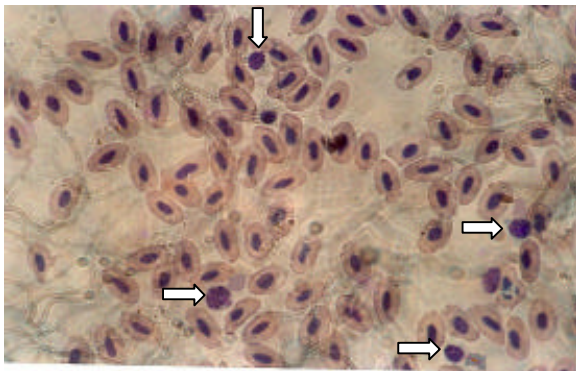


Fig. 2: Photomicrograph showing different sizes of lymphocytes (arrows) in Muscovy ducks x100

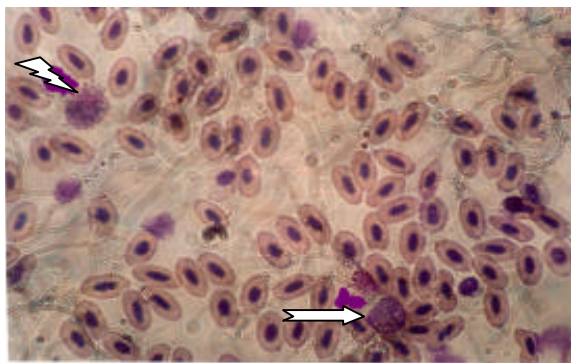


Fig. 3: Photomicrograph showing the Basophil (lightning bolt arrow) and Monocyte (notched left arrow) of Muscovy ducks x100

The monocytes counts showed that the male Muscovy ducks had a similar average monocytes count of 8.25% with the female Muscovy ducks 8.75% (Table 2). The monocytes were observed to be similar to the lymphocytes though larger than the lymphocytes. The

nucleus of the monocytes was kidney shaped or oval and the cells had light basophilic cytoplasm (Fig. 3 notched left arrow).

DISCUSSION

In this study, we did not experience much difficulty during the diluting and counting of the erythrocytes, while the leucocyte diluent used, Brilliant cresyl blue solution (Rees-Eckers solution) did give a fairly good differentiation of the leucocytes after 15-20 min as been reported in the literature (Lucas and Jamroz, 1961). The erythrocytes appear elongated or elliptical while the leucocytes were oval or round in shape.

The erythrocytes of the Muscovy ducks were oval with an orange-blue or greyish pink cytoplasm. The nucleus was either oval, round or elongated staining dark blue or purple. These characteristic displayed by the erythrocytes of the Muscovy ducks were similar to those described for the domestic fowl (Bell and Freeman, 1971).

Average total erythrocyte counts obtained for the Muscovy ducks was lower than those for temperate ducks. The Muscovy ducks was lower than the Nigerian domestic chicken as reported (Oyewale, 1987). This differences in counts may be attributed to the hot humid climate of the tropics in which the Muscovy ducks exist which may depress the erythrocyte production as suggested for difference in erythrocyte counts between commercial layer breeds of chicken in Ghana compared to temperate breeds of chickens (Awotowi, 1991).

The male Muscovy ducks were observed to have a higher erythrocyte count than female Muscovy ducks. This may have been attributed to hormonal influence (Androgen) in the males. It has been suggested that Androgen hormone plays a part in alteration of erythrocyte and some blood cell numbers (Juhn and Domm, 1930).

The average total leucocyte counts obtained for the Muscovy ducks in this study was higher than those for temperate breed of ducks. Earlier studies (Gilbert, 1965) reported an average count of 28,000 per mm³ while a much earlier study (Magath and Giggins, 1934) reported an average of 25,300 leucocytes for 34 ducks. However in this study, an average count of 31,000 cells per mm³ was obtained. This difference may be attributed to the diversity in system of management, breed and environmental influences as proposed in a similar study (Chubb and Rowell, 1959).

There was no significant difference in the total average leucocyte count of male Muscovy ducks with the female Muscovy. The Nigerian domestic chickens have a far lower average leucocyte count of 7.98 ± 0.88 (Oyewale, 1987) compared to the average count obtained for the Muscovy ducks. This may be attributed to specie difference as suggested in an earlier study (Roger and Carlton, 1967).

The heterophils were observed to have rod-like granules, staining pink and the cell outline was observed to be irregular. The heterophils being a polymorphonuclear cell shows variation in lobulation of their nucleus. It was noted that the best criteria to distinguish the heterophils from the esinophils is by using the rod or spindle shaped intra-cytoplasmic granule of the heterophils rather than the staining characteristics as described earlier (Bell and Freeman, 1971).

There were no appreciable differences in average counts of the heterophils between the male and female sexes of the Muscovy ducks contrary to the higher counts obtained for male domestic chicken (Oyewale, 1987).

The heterophils of the Muscovy ducks as in domestic chicken were second in population after the lymphocytes. However, the heterophils average count obtained (18.87%) was far lower than that recorded for Mallard ducks decades ago (Magath and Giggins, 1934).

The esinophils of the Muscovy ducks were observed to have spherical granules which were eosinophilic in staining with a distinct cellular outline (ovoid) as against the irregular cell outline of the heterophils. It was observed that the best way of identifying the esinophils is by using their granular shape, distinguishing them from the heterophils.

The nucleus of some of the esinophils has been noted to be multilobulated while some were bilobed. This goes contrary to the observation of Olson (1937) in which he described the nucleus of the esinophils as only bilobed.

The esinophils average count obtained from the Muscovy ducks was observed to be lower than those reported for temperate breeds of ducks as supported by earlier studies (Lucas and Jamroz, 1961) in which it reported an average count of 7% for the Mallard ducks. These differences may be due to management

practices, breed disposition or environmental factors as put forward by other workers (Chubb and Rowell, 1959). However, the average 4% esinophils count obtained for the Muscovy ducks was slightly higher than the 2% average count reported for domestic (Bell and Freeman, 1971). Furthermore, it has also been reported that the esinophils of Muscovy ducks was greater than that reported for Nigerian domestic chicken (Oyewale, 1987). Importantly, there was no significant difference between the male and female Muscovy esinophils counts.

The basophils of the Muscovy ducks were observed to be ovoid with a slightly basophilic to colourless cytoplasm with strongly basophilic granules which in some of the cells mask the nucleus. This unique characteristic display of the basophils in Muscovy ducks coincides with those described for the domestic fowl (Bell and Freeman, 1971). The average basophilic count for the Muscovy duck (1.12%) falls within the range of 0.0-4% as suggested in an earlier study (Magath and Giggins, 1934) but was found to be lower than the 5% average count as reported in another study (Lucas and Jamroz, 1961).

The female Muscovy ducks also had a higher (1.5%) average basophil count than the males (0.75%). This may be attributed to hormonal influence particularly as it is consistent with the observation of Oyewale (1987), for the Nigerian domestic chicken.

The monocytes of the Muscovy ducks in this study were observed to be similar to the large lymphocytes as had been previously observed (Olson, 1937). Their nucleus is kidney shaped or oval with a light basophilic cytoplasm. Average monocytes count (8.67%) obtained for the Muscovy duck was found to be in agreement with an earlier reports (Lucas and Jamroz, 1961) for adult Mallard ducks (8%) and within the 4-20% range reported in another study (Magath and Giggins, 1934). There was however no significant sex difference in the monocytes counts of the Muscovy ducks.

The lymphocytes of the Muscovy ducks were observed to have variable sizes and this is consistent with an earlier study (Lucas and Jamroz, 1961). It is therefore logical to avoid classifying the lymphocytes into small and large lymphocytes as suggested by some workers (Lucas and Jamroz, 1961) since a clear-cut size can not be established for the two cell categories. The nucleus of the lymphocyte was large, filling the whole cell and was basophilic while the small rim of cytoplasm was pale blue.

The average count obtained for the lymphocyte in this study was observed to be more than all the leucocytes put together (granular and agranular leucocytes). This observation is consistent with those reported for the domestic fowl (Oyewale, 1987). The average lymphocyte count agrees with those counted in an earlier study (Magath and Giggins, 1934) However, the lymphocyte count was far greater than those recorded for the Mallard

ducks (32%) as reported in an earlier studies (Lucas and Jamroz, 1961). Average lymphocyte count for the male Muscovy duck (72.75%) was higher than that of the female Muscovy ducks (61.37%).

In conclusion, we report here in that the Muscovy duck have a lower average erythrocyte count compared to temperate breed of ducks but has higher erythrocyte count than the Nigerian domestic chickens. The male Muscovy ducks have a higher erythrocytes count than the female Muscovy ducks. Furthermore the Muscovy ducks have a higher total leucocyte count than the temperate breed of ducks and the Nigerian domestic chickens. Additionally, the Muscovy ducks have more heterophils and esinophils than temperate breed of ducks. Indeed, the male Muscovy ducks have a higher average lymphocyte count than the females but have a lower basophil count as compared to the female Muscovy ducks. The esinophils were found to have diverse number of lobes of their nucleus rather than being only bilobed.

On a final note, management practices, breed and species differences and environmental factors in one way or the other, may have an influence in the blood picture of the different domesticated birds.

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