Seasonal Variation in the Haematological Parameters of the Adult Mallard Duck in a Tropical Environment

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Authors’ contributions

This work was carried out in collaboration among all authors. Author OSO carried out the experiment and wrote the first draft. Author OIA carried out part of the laboratory work, the statistical analysis and corrected the manuscript while author JOO designed and supervised the study. All authors read and approved the final manuscript.

ABSTRACT

Duck production is a growing poultry enterprise in Nigeria and they are mostly reared in extensive management system. However, the haematological profiles as influenced by the tropical environment have not been well documented. The objective of the present study was to examine the seasonal variation in the haematological parameters of the adult Mallard duck in the tropical environment of Nigeria; as they effects duck production adversely. The Erythrocyte, leucocyte and platelet counts, as well as the erythrocyte osmotic fragility of the domestic duck of the mallard breed during the wet and dry seasons in the hot humid tropical environment of the Experimental Animal Unit of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria was investigated.

The study showed that the values of the packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and platelet were significantly higher in the dry season than in the wet season, but the red blood cell (RBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total and differential leucocyte values were similar in the two seasons. The erythrocyte fragility was also higher in the dry season.

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In conclusion the higher PCV, MCH and platelet values in the dry season might have resulted from haemoconcentration occasioned by higher evaporative heat loss, which is a common occurrence in the dry season. The higher erythrocyte fragility could have been the result of stress induced by the high ambient temperature during the dry season, or higher metabolic rate associated with lactic acid accumulation, which has been shown to increase erythrocyte osmotic fragility.

Keywords: Season; haematology; erythrocyte osmotic fragility; mallard duck.

1. INTRODUCTION

One of the most serious nutritional problems in the developing countries (Nigeria inclusive) is the shortage of high protein food from animal sources. One of such potential source of animal protein, which is not popularly produced in Nigeria, is the duck [1]. Among the different species of ducks reared in Nigeria like Muscovy duck, Mallard and Pekin, Mallard ducks are better layers than Muscovy ducks and can produce up to 300 eggs per year [2]. Analysis of normal haematological parameters of domestic animal is very essential in diagnosing the various pathological and metabolic disorders [3]. According to Etim et al. [3], haematological parameters and their variations are routinely used to determine the health status of the body and to determine stresses due to environmental, nutritional and / or pathological factors. Many of these factors are elevated at particular times of the year in form of seasonal changes resulting in low egg and meat production. This is especially important in Mallard ducks, which are new entries in poultry and exotic birds production in Nigeria, after their introduction from Europe [4]. Several studies have been carried out on haematological parameters of mallard ducks in temperate environment [5]. However, few reports are available on this avian species in the hot humid tropics, where the bird, in recent times, is becoming increasingly popular as an alternative source of animal protein. Although they are not as many as Muscovy, it is believed that the mallard ducks lay more eggs than Muscovy ducks, producing up to 300 eggs per annum [6]. Recently, Oladele et al. [7] reported the baseline haematological data of this bird in the hot humid environment. Haematological parameters in animals and man have been widely reported to be influenced by several environmental factors. Among such factors are ambient temperature, relative humidity, time of the day and season of the year, which have all been shown to contribute to the variations seen in these physiological parameters [8]. However, there have been several conflicting reports on the effects of season on the haematological parameters in various animal species. For example, Olayemi and Arowolo [9] reported higher red blood cell (RBC), packed cell volume (PCV), total WBC and lymphocyte values in the wet than in the dry season in the Nigerian local duck.

Seasonal variation in the haematological parameters of the adult Mallard duck in a tropical environment have a huge impact on duck production, keeping this in view, the present study was undertaken to determine whether the haematological parameters of the mallard duck varied between the dry and the wet seasons in the study area.

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of seventeen (17) adult mallard duck of about 2 years old, weighing 1.5 – 2 kg were procured from a local market in Ibadan, South West Nigeria, and kept at the Experimental Animal Unit of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria. The birds were allowed to acclimatize for a period of two (2) weeks, during which they were dewormed using piperazine dihydrate (Alfasan, Holland) at 0.1 g/L of drinking water for one day. The birds were fed with maize waste in wet slurry form with guinea corn incorporated and clean portable water was also provided ad libitum throughout the period of the experiment.

2.2 Sample Collection

Blood (5 ml) was collected from each bird through the right jugular vein into heparinized bottles in October, 2008 during the wet season and in February, 2009 during the hot dry season and were analyzed immediately. Meteorological data were also obtained for the period from the Nigeria Meteorological Service Station, Ibadan, Oyo State.
2.3 Determination of Haematological Parameters

From the blood samples, the packed cell volume (PCV) was determined by the microhaematocrit method. Red blood cells (RBC) and white blood cells (WBC) were counted using the improved Neubauer haemocytometer [10]. Haemoglobin concentration (Hb) was determined by the cyanmethaemoglobin method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values of PCV, RBC and Hb as described by Jain [10]. Fresh blood smear was fixed with methanol and stained with Giemsa for differential leucocyte count. Erythrocyte osmotic fragility was determined according to the method of Oyewale [11] as briefly described below.

2.4 Packed Cell Volume

From the blood samples collected, the PCV was determined using the microhaematocrit method as described by Jain [10]. Using this method, blood was drawn into the plain capillary tube by capillary traction to three-quarter of its length. The capillary tube was tipped to permit the blood to flow toward the free end to provide sufficient space to prevent outflow when the opposite end was sealed. The outside of the capillary tube was wiped free of blood. As the index finger was placed over the moist end to hold the column of blood in place, as the opposite dry end was forced into the sealing material (plasticine) to form a tight plug. The capillary tube was placed in a microhaematocrit centrifuge (Hawkskey, Gelman Instruments, England) with the sealed end pointing outward. The blood was then centrifuged for 5 minutes at a speed of 3,000 revolutions per minute. PCV was then determined using a graphic reading scale.

2.5 Haemoglobin Concentration

Drabkin’s solution (4 ml) was carefully transferred to a clean test tube and 0.02 ml of blood was added and allowed to stand for at least 20 minutes. The optical density (OD) of the mixture placed in a cuvette was then read using spectrophotometer (SP 6100 model, Jenway, England) at a wavelength of 540 nm. Similar 4 ml of Drabkin’s solution was used as the blank. The OD of an Hb standard of known concentration was then determined and the value used to calculate the Hb concentration in the sample, thus:

\[ \text{Haemoglobin (Hb) concentration} = \frac{\text{OD of blood}}{\text{OD of standard}} \times \text{Hb concentration of standard} \]

Where OD = Optical density

2.6 Red Blood Cell Count

The RBC was counted made in a haemocytometer using avian diluting fluid also known as Hayem’s solution (0.5 mercuric chloride, 1.0 g sodium chloride, 5 g Sodium Sulphate and 200 ml distilled water). The number of erythrocytes in 5 of the 25 squares in the central area of each chamber of the Neubauer haemocytometer was counted, taking the four corner squares and the central one.

The total number of erythrocytes obtained was multiplied by depth (x10), area (x5), and dilution factor (x200). Hence, if Y erythrocytes had been counted, the number of erythrocytes per microlitre of blood in the original sample would be 10,000 Y.

2.7 Total White Blood Cell Count

The total WBC count was determined using the haemocytometer method. Blood was filled up to 0.5 mark on the WBC pipette in the haemocytometer. This was also filled with WBC up to 1.1 mark on the pipette. The WBCs were allowed to settle for one minute, mixed thoroughly and the Neubauer’s slide was filled with the WBC diluting fluid (2 ml glacial acetic acid, 1 ml of 1% gentian violet and 0.1N HCl all in 100 ml of distilled water). Total WBCs were counted in the 16 square in the corner of the slide. Finally, total WBCs counted were then multiplied by 50 (number of cells counted x20 (dilution) x10 (depth)) divided by 4 (no of squares).

2.8 Differential Leucocyte Count

A fresh smear of each blood sample was prepared and fixed with methanol and then stained with Giemsa. One hundred cells were each identified morphologically and counted and the number of each WBC type was expressed as a percentage of the total WBC.

2.9 Erythrocyte Osmotic Fragility

Erythrocyte osmotic fragility was determined according to the method described by Oyewale
Briefly 0.02 ml of blood was added to tubes containing increasing concentration of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 (0, 0.1, 0.2, 0.3, 0.5, 0.7, 0.8 and 0.9%). The tubes were gently mixed and incubated at room temperature (29°C) for 30 minutes. The content of each tube was then centrifuged at 3500 rev/min for 10 minutes and the supernatant decanted. The optical density (OD) of the supernatant was determined spectrophotometrically at 540 nm using SM22PC Spectrophotometer (Surgienfield Instruments, England). Haemolysis in each tube was expressed as a percentage, taking haemolysis in distilled water (0% NaCl) as 100%.

2.10 Data Analysis

Data obtained were presented as means ± SEM and were compared between seasons using student’s t-test. P value less than 0.05 was considered significant.

3. RESULTS

The meteorological data obtained during the period of study in Ibadan (longitude 3°53′47” East and latitude 7°23′16” north of the equator) is shown in Table 1.

Tables 2 and 3 present the erythrocyte and leucocyte values of the Mallard duck during the wet and dry seasons. Except for the PCV and MCH values which were significantly higher (P<0.05) in the dry season, all the other erythrocyte values of the Mallard duck did not show significant seasonal variation. Similarly, the leucocytes values (total WBC counts and lymphocyte, heterophil, eosinophil and monocyte counts) did not show any significant difference in the ducks between the wet and dry seasons (Table 3). However, the thrombocyte (platelet) count was significantly higher (P<0.05) in the dry season.

<table>
<thead>
<tr>
<th>Period</th>
<th>Date</th>
<th>Temperature (°C)</th>
<th>Relative humidity (%)</th>
<th>Rainfall (mm)</th>
<th>Sunshine (watt/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet Season</td>
<td>Oct. 5 - Nov 5, 2008</td>
<td>31.59 ± 0.39</td>
<td>78.67 ± 3.49</td>
<td>6.87 ± 3.26</td>
<td>6.44 ± 0.41</td>
</tr>
<tr>
<td>Dry Season</td>
<td>Jan. 5 - Feb 5, 2009</td>
<td>32.74 ± 0.77</td>
<td>69.73 ± 3.92</td>
<td>0.43 ± 0.07</td>
<td>4.81 ± 0.75</td>
</tr>
</tbody>
</table>

**Table 2. Erythrocyte values (mean ± SEM) of the adult mallard duck during wet and dry seasons in the hot humid tropics**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Wet season (n=17)</th>
<th>Dry season (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>33.12 ± 0.81</td>
<td>35.07 ± 0.74*</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.20 ± 0.38</td>
<td>11.65 ± 0.25</td>
</tr>
<tr>
<td>RBC (x 10⁶/µl)</td>
<td>3.49 ± 0.09</td>
<td>3.41 ± 0.12</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>95.08 ± 1.50</td>
<td>104.93 ± 5.02</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>32.10 ± 0.73</td>
<td>35.11 ± 1.67*</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.75 ± 0.50</td>
<td>33.68 ± 0.47</td>
</tr>
</tbody>
</table>

**n= Number of birds.**

*Significantly different from the values at wet season

**Table 3. Leucocyte and platelet values (mean ± SEM) of the adult mallard duck during wet and dry season**

<table>
<thead>
<tr>
<th>Parameters (x 10³/µl)</th>
<th>Wet season (n=17)</th>
<th>Dry season (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC</td>
<td>15.04 ± 0.63</td>
<td>14.86 ± 0.32</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>9.80 ± 0.65 (69.71 ± 1.55%)</td>
<td>10.73 ± 0.39 (72.00 ± 1.47%)</td>
</tr>
<tr>
<td>Heterophil</td>
<td>4.10 ± 0.36 (26.59 ± 1.61%)</td>
<td>3.62 ± 0.21 (24.53 ± 1.52%)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.33 ± 0.06 (1.81 ± 0.23%)</td>
<td>0.24 ± 0.03 (1.60 ± 0.24%)</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0.34 ± 0.06 (2.40 ± 0.45%)</td>
<td>0.27 ± 0.05 (1.86 ± 0.35%)</td>
</tr>
<tr>
<td>Platelets</td>
<td>118.94 ± 4.35</td>
<td>140.60 ± 7.35*</td>
</tr>
</tbody>
</table>

**n= Number of birds.**

Values in parentheses are the relative leucocyte counts expressed in percent

*Significantly different from the value at wet season
Fig. 1. Erythrocyte osmotic fragility of the mallard duck during the wet and dry season

Values are mean±SEM

* Significantly different (P<0.05) from the value at wet season

As shown in Fig. 1, the erythrocyte osmotic fragility of the duck in the wet season at the 0.7% NaCl concentration was significantly lower (P<0.05) than the corresponding value obtained in the dry season. The fragility values were however, not significantly different at all the other NaCl concentrations.

4. DISCUSSION

This present study showed that season has some effects, though not remarkable, on the haematological parameters of the mallard duck in the tropics. It was considered not remarkable because, of all the erythrocyte values investigated, only the PCV and the MCH values showed significant variations, with their values being higher in the dry than in the wet season. The platelet count was also higher in the dry season, while the RBC, Hb, MCV and MCHC did not show any seasonal differences. The higher PCV, MCH and platelet values in the dry season may be due to haemoconcentration produced as a result of higher evaporative water loss during the dry season. It has been widely reported that relative humidity, which is usually lower in the dry season results in elevated evaporative water loss because body water is converted to vapour which quickly lost to the environment during this period. Similarly, Pavlak et al. [12], on the other hand, found that in pigeons the PCV, Hb, MCV, MCH and WBC values were lower in the winter than in the summer in the temperate region of Zagreb, Croatia. They suggested that the variation was also due to fall in plasma volume with the resultant haemoconcentration. Contrary to our findings, Oladele et al. [13] reported higher PCV and Hb values in the wet season than in the dry season in pigeons in Zaria, Northern Nigeria. Therefore, the relationship between evaporative water loss and relative humidity in small animals and bird is inverse and linear [14]. Coopers and Withers, [15] in a study on adaptation of Marsupials to changes in micro environmental conditions also reported that animals lose more body water in the form of evaporative water loss in drier ambient condition when the humidity is low.

The total and differential leucocyte values did not exhibit any seasonal variation in the mallard ducks. Since leukocytosis is a function of exposure to pathogens [16], this indicates that body resistance to infection is similar and constant throughout the year in the mallard duck without challenge from any pathogen. This lack of considerable seasonal variation may be due to the fact that the birds were maintained on the same regular diet throughout period of the experiment, rather than being gathered from the wild at each of the season. Unlike previous reports on the Nigerian local duck studied in the same environment. These were shown to exhibit considerable seasonal variation in the PCV,
RBC, MCV, MCH and MCHC values [9]. It is noteworthy however; that the PCV, Hb, RBC, MCV, MCH and MCHC values obtained in the present study was lower than those of the Nigeria local duck. This difference may be of genetic origin because variations in haematological parameters have been reported among different strains of the domestic chicken [17,18,19,20,21] and in the duck [22]. Erythrocyte values of the mallard ducks obtained in the present study during the wet season in September were also lower than the values reported in mallard ducks in the temperate environment at the same period of the year by Shave and Howard [22]. This of course must be due to the climatic conditions, which the birds were subjected or exposed to, because low ambient temperature would reduce water intake, resulting in haemoconcentration.

The PCV, MCH and the platelet values that were higher in the dry than in the wet season in this study might not be unconnected with haemoconcentration too as suggested by Olayemi and Arowolo [9]. The haemoconcentration must have been occasioned by higher evaporative heat loss due to higher ambient temperature coupled with considerably lower relative humidity during the dry season in the tropics (Table 1).

Erythrocyte osmotic fragility test has been reported to be an indicator of stress by several authors. Such stress factors such as ageing, exhaustive exercise and oxidative stress have been associated with higher erythrocyte fragility in man and animals [23,24,25,26]. Thus, higher erythrocyte osmotic fragility in the mallard duck during the dry season in this study showed that the birds were stressed during the dry season. Although, the plasma corticosterone levels were not determined, the high ambient temperature and lactic acid build up as a result of increased metabolic activities during the dry season must have led to increased fluidity of the erythrocyte membrane which will definitely decrease erythrocyte osmotic resistance. Owewale, [11] had previously reported that increased temperature and decreased pH of the medium increased erythrocyte fragility in the domestic chicken. Similarly, Hanzawa and Watanabe [27] also reported that accumulation of lactic acid during anaerobic exercise increased erythrocyte fragility in thorough bred horse. High ambient, temperature may also increase erythrocyte fragility [28] as result of increased rate of generation of free radical, resulting in lipid peroxidation and damage to erythrocyte membrane proteins [25].

5. CONCLUSION

This study has shown that the PCV, MCH, platelet values and erythrocyte fragility in the mallard duck were higher in the dry season than in the wet. We can therefore conclude that increased evaporative water loss associated with lower relative humidity and higher ambient temperature observable in the hot humid tropics during the dry season may influence some haematological parameters and erythrocyte membrane stability in the mallard duck. This must therefore be taken into consideration when haematological data is being used for evaluation of health status in these birds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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