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# Erythrocyte Osmotic Fragility of Nera Black Fowls of Two - Age Groups

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**Abstract:** The erythrocyte osmotic fragility and other erythrocyte indices in fowls of two different age groups (7-9 week-old and 49 week-old) were studied using Nera Black strain usually raised commercially for egg production in Nigeria. Erythrocytes in the 49 week-old birds were more fragile than those in the 7-9 week-old at sodium chloride (NaCl) concentrations of 0.2% ($p<0.05$), 0.7% ($p<0.05$), 0.8% ($p<0.01$) and 0.9% ($p<0.01$). The mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCH) values were also higher in the adults, but the packed cell volume (PCV) was lower. The haemoglobin (Hb) concentration, red blood cell (RBC) count and mean corpuscular volume (MCV) were similar in the two age groups.

**Key words:** Osmotic fragility, erythrocytes, nera black fowl and age

## INTRODUCTION

The effect of age on osmotic fragility of erythrocytes has been studied in humans (Magnani *et al*., 1988; Srour *et al*., 2000; Tiffert *et al*., 2007), rats (Ajit and Rukmini, 1991; Jayachandram *et al*., 1996), rabbits (Davies and Goldberg, 1987; Brzezinska-Slebodzinska, 2001), guinea-fowl (Oyewale, 1991) and turkeys (Oyewale and Ajibade, 1990). It has been shown that erythrocyte osmotic fragility increases with age in humans (Srour *et al*., 2000; Meurs *et al*., 2005) and in guinea-fowl (*Numida maleagris*) (Oyewale, 1991). However, in white England turkeys, Oyewale and Ajibade (1990) reported that erythrocytes of the 6-8 weeks old were more fragile than those of the 20-22 week-old. Brzezinska - Slebodzinska (2001) also observed that erythrocytes in younger rabbits (1.5 months) are more susceptible to membrane peroxidation than those of 3.5 and 6 months old rabbits. Studies in our laboratory on the domestic fowl have shown that the osmotic fragility of erythrocytes varies between sexes and breeds (Oyewale and Durotoye, 1988) and at different temperatures and pH (Oyewale, 1992). It is of interest to examine in the present study whether differences exist in the osmotic fragility of erythrocytes of the young and adult Nera black fowls in the hot humid tropical environment.

Nera Black fowl is a dual purpose bird raised commercially in our environment for meat and egg production. They are so called because of their hardiness, high growth rate, large egg size and high egg production rate.

## MATERIALS AND METHODS

Twenty young female (7-9 weeks old) and 19 adult female (49 weeks old) domestic fowls of the Nera Black strain were used for this study. The birds were intensively reared at the University of Ibadan Teaching and Research farm, where they received balanced poultry diet (Capsfeed Ltd, Ibadan, Nigeria) and water *ad-libitum*.

Blood samples were obtained from each bird into bottles containing ethylene diamine tetra acetic acid (EDTA) (2mg/ml of blood) and analyzed immediately. The red blood cell (RBC) count was determined by the haemocytometer method, the packed cell volume (PCV) by the microhaematocrit method and the haemoglobin (Hb) concentration by the cyanmethaemoglobin method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values obtained for RBC, PCV and Hb according to Jain (1986).

The erythrocyte osmotic fragility was determined as described by Oyewale (1992). 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 for 30 minutes. The content in each tube was then centrifuged at 1500 rev/ min for 10 minutes and supernatant decanted. Optical density of the supernatant was determined spectrophotometrically at 540nm. Haemolysis in each tube was expressed as a percentage, taking haemolysis in distilled water (0% NaCl) as 100%.

The results obtained are expressed as mean ± standard deviation (SD) and compared using Student's t-test.

## RESULTS

As shown in Fig. 1, the osmotic fragility of erythrocytes of the Nera black fowl was significantly higher in the 49 week-old than in the 7-9 week-old at NaCl concentrations of 0.2% ($p<0.05$), 0.7% ($p<0.05$), 0.8%

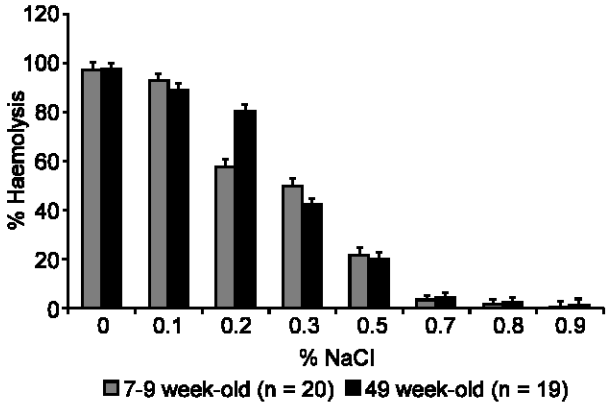


Fig 1: Erythrocyte osmotic fragility in 7-9 week-old and 49 week-old Nera black fowls.

(p<0.01) and 0.9% (p<0.01). The osmotic fragility was not significantly different between the two groups of birds at all the other NaCl concentrations.

Table 1 shows the haematological values in the young (7-9 weeks old) and adult (49 week-old) Nera Black fowls. The PCV was significantly higher (P< 0.01) in the young fowls than in the adults. However, the MCH and MCHC values were significantly lower (P< 0.05 and P<0.01 respectively) in the young birds. On the other hand, the Hb, RBC and MCV values were similar in the two-age groups.

Table 2 shows a comparison of the erythrocyte parameters obtained in Nera Black fowls in the present study, with those reported by Oyewale and Durotoye (1988) in adult Nigerian domestic fowls and adult Hubbard fowls in the same humid tropical environment. The PCV and Hb values in Nera Black fowls were significantly lower than those of Nigerian domestic fowl (P<0.01) and Hubbard fowls (P<0.01). However, the MCV in the Nera Black was similar to those of the Nigerian fowl and Hubbard fowl. The RBC count of the Nera Black fowl was also similar to that of the Hubbard fowl, but was lower than that of the Nigerian domestic fowls.

Table 1: Haematological values (mean±SD) of 7-9 week-old and 49 week-old Nera black fowls

| | 7-9 week-old (n=20) | 49 week-old (n=19) |
|---|---|---|
| RBC x $10^6$/µL | 1.86±0.36 | 1.81±0.44 |
| PCV % | 26.45±3.80 | 22.30±2.70* |
| Hb g/dl | 4.42±1.20 | 5.00±2.10 |
| MCV fl | 145.74±39.80 | 127.00±35.5 |
| MCH pg | 24.80±9.50 | 32.01±11.09** |
| MCHC g/dl | 17.11±5.27 | 26.06±10.08* |

n = Number of birds. Asterisks indicate significant difference from values at 7-9 week-old (* = p<0.01; ** = p<0.05).

Table 2: Comparison of erythrocyte parameters of Nera Black fowl (present study), and Nigerian domestic and Hubbard fowls (Oyewale and Durotoye, 1988)

| | Nera Black fowls (n=19) | Nigerian domestic fowl (n=13) | Hubbard Fowl (n=12) |
|---|---|---|---|
| PCV % | 22.30±2.70* | 33.39±1.70 | 28.29±2.16 |
| RBC X $10^6$ µL | 1.81±0.44* | 2.42±0.38 | 2.14±0.25 |
| Hb g/dl | 5.00±2.10* | 9.84±1.0 | 9.19±0.65 |
| MCV fl | 127.00±35.50 | 137.23±22.15 | 133.08±11.67 |
| MCH pg | 32.01±11.09 | 40.66 | 42.94 |
| MCHC g/dl | 26.06±10.08 | 29.47 | 32.48 |

n = Number of birds. Values are means ± SD, except where mean value only is available. Asterisk indicates significant difference (*=P<0.01) from Nigerian domestic fowl or Hubbard fowl.

## DISCUSSION

From the present study, the erythrocyte osmotic fragility was higher in the older birds than in the younger ones. This is similar to the observation of Oyewale (1991) in guinea-fowl (*Numida maleagris*), in which erythrocytes of 156- week-old birds were more fragile than those of 21- week-old. In contrast, Oyewale and Ajibade (1990) reported that erythrocytes of the 6-9 week-old turkey were more fragile than those of the 20-22 week-old. Increased osmotic fragility has also been associated with increasing age in rats (Jayachandram *et al*., 1996), rabbits (Davies and Goldberg, 1987) and human erythrocytes (Srour *et al*., 2000; Tilfert *et al*., 2007). Davies and Goldberg (1987) associated the increase in osmotic fragility with age with the damage of erythrocytes membrane by oxygen radicals in the form of lipid peroxidation and protein degradation. This was further supported by Droge, (2002) who demonstrated that reactive oxygen species and other free radicals generated as animals advance in age contribute to protein degradation, lipid peroxidation, DNA oxidation, muscle wasting and mitochondria DNA damage. Replicative senescence, telomere shortening and cellular apoptosis, all seen during the aging process have also been associated with increased oxidative damage by free radicals and reactive oxygen species (Von Zglinicki *et al*., 1995; Sitte *et al*., 2000). Tilfert *et al*. (2007) however, reported that increased osmotic fragility of erythrocyte during aging is due to reduction in the number and activities of calcium mediated potassium channels in the erythrocyte membrane.

The observation of a higher PCV in the young birds than in the adult in the present study is contrary to the report of Islam *et al*. (2004), who observed increases in PCV in Fayoumi, Assil and local Bangladesh chicken following increasing age. Also, PCV has been shown to increase with age in ring-necked pheasants *(Phasianus colchicus)* (Schmidt *et al*., 2007), pigeon (*Columba livia domestica*) (Pavlak *et al*., 2005) and in captive reared wild birds such as houbara, white bellied and rufous-crested bustards (Howlett *et al*., 2002). Furthermore, Oyewale and Ajibade (1990) found a lower PCV in 6-8 week-old turkeys than in the 22 week-old, but no difference was observed in the PCV of guinea fowls of two age groups (21 and 156 weeks of age) (Oyewale, 1991).

The lower MCHC values obtained in the younger Nera black fowls than in the adult is in conformity with the observations in Japanese quail (*Cortunix cortunix japonica)* (Nirmalan and Robinson, 1971) and Fayoumi birds (Islam *et al.,* 2004). According to Nirmalan and Robinson (1971), this may have been caused by stimulation of bone marrow by erythropoietin released consequent to the hypoxia associated with rapid growth and high metabolic rate.

The PCV, RBC and Hb values were significantly lower in the Nera Black fowls than in Hubbard and Nigerian domestic fowls (Table 2). This is more likely due to genetic than dietary influences, because even in Hubbard and Nigerian domestic fowl that received the same diet, these parameters were found to be lower in the former than in the latter (Oyewale and Durotoye, 1988).

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