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Effect of Battery and Furnished Cages on Blood Viscosity and Erythrocyte Osmotic Stability of Laying Hens

Oluwapelumi V. Boluwaji
Lecturer, Department of Animal Production and Health,
Federal University, Oye-Ekiti, Nigeria
Samuel O. Aro
Professor, Department of Animal Production and Health,
Federal University of Technology, Akure, Nigeria
Clifford A. Chineke
Professor, Department of Animal Production and Health,
Federal University of Technology, Akure, Nigeria

Abstract:
As a result of animal welfare issues, the use of furnished cages has been proposed. Blood viscosity and osmotic stability can be used as an indication of oxidative stress. There has, however, not been any study investigating the blood viscosity and erythrocyte osmotic stability of laying hens reared in battery and furnished cages, hence the reason for this experiment. 790 laying hens were reared in large and small furnished cages and battery cages between 14 and 60 weeks. The whole blood viscosity and erythrocyte osmotic stability were determined at the end of the experiment. The study showed that there was no significant difference in the whole blood viscosity of laying hens reared in the three cage types, while hens reared in battery cages had the highest number of stable red blood cells. This could be an indication that the stress level in the three cages is similar, while birds are more osmotically stable in the battery cages.

Keywords: *Blood, red blood cells, poultry birds, osmotic stability, cages*

1. Introduction

In the commercial poultry industry, in Nigeria and globally, layers are primarily housed in battery or conventional cages. All over the world, many welfare issues have been associated with birds raised in battery cages (Dawkins et al., 2004). Birds prefer to perform certain natural behaviors, such as dustbathing and nesting. However, within the battery cages, the birds' ability to perform the behaviours is restricted and bone quality is reduced due to the barren environment (Fleming et al., 1994; Vestergaard et al., 1997; Tauson, 1998). Due to this, there is growing pressure from animal welfare groups advocating the ban on battery cage systems in the poultry industry. This led to the introduction of legislation that banned battery cages in Europe (CEC, 1998, 1999). As a result of the ban, several alternative systems have been proposed and practiced in the last decade (Tauson, 2004; Hester, 2005; Mertens et al., 2006). Among the alternative system is the furnished (also called enriched or modified) cage that seems to offer a more suitable housing system for improved welfare of birds (FAWC, 2007). The cages are equipped with perches, dust baths, and nests, allowing the birds to perform their natural behaviors, such as nesting and roosting (Lindberg & Nicol, 1997; Newberry, 1999; Cordiner & Savory, 2000). Previous studies have also shown that birds housed in furnished cages improve hen welfare by reducing fear, aggression, and feather pecking and increasing bone mineral density (Gvaryahu et al., 1994; Newberry, 1995; Kopka et al., 2003; Vits et al., 2005). Also, birds raised in an environment that is furnished had lower aggression, lower body weight, and lower corticosterone levels than the birds raised in battery cages (Pavlik et al., 2007). Barnett et al. (2009) found that group size and living space had little effect on layer welfare, while cage equipment (perch, sand bath, and nest) had no influence on bird welfare. However, it had a positive effect on bone strength, although leg deformations were present in layers kept in furnished cages, probably due to excessive perch use, which may be a problem in this type of cage (Vits et al., 2005). However, no study has been conducted to investigate the effect of furnished cages on blood viscosity and erythrocyte osmotic stability of laying hens.

Blood viscosity is a measure of the thickness or stickiness of blood. It is a direct measure of the ability of blood to flow through the blood vessels. Increased blood viscosity can be caused by an increase in red cell mass or increased red cell deformity, increased plasma levels of fibrinogen and coagulation factors, as well as dehydration (Aro, 2014; Aro & Akinlemimu, 2015; Aro, 2018; Aro et al., 2018). Erythrocyte osmotic fragility (EOF) is an important biomarker of oxidative stress (Minka & Ayo, 2010a; Asala et al., 2011). In birds, it is used to assess the stability of erythrocytes in hypotonic solutions (Minka & Ayo, 2010a; 2013) by estimating haemolysis under hypoosmotic stress. An increased haemolysis

shows an increase in osmotic fragility or a decrease in osmotic stability in an inverse corollary (Igbokwe, 2016). Erythrocyte osmotic fragility test can be used to assess transportation stress (Minka & Ayo, 2010b) and vitamin E status (Pillai et al., 1992). Other extrinsic factors which affect erythrocyte osmotic fragility include:

- Temperature,
- pH of an isotonic solution,
- Osmolarity, and
- Type of media,
- Oxygenation,
- Season,
- Ionic strength of media, and
- Medicinal plants and drugs (Chikezie, 2007; Oyewale et al., 2011; Habibu et al., 2016; Islah et al., 2016; Igbokwe & Igbokwe, 2016a; Igbokwe & Igbokwe, 2016b)

Intrinsic factors such as age, genes, species, breed, phenotype, gender, pregnancy and lactation, egg laying, size, and differences in erythrocyte membrane composition (Habibu et al., 2013; Habibu et al., 2014; Igbokwe et al., 2015a; Igbokwe et al., 2015b; Igbokwe et al., 2016c) can also affect the osmotic fragility of erythrocytes. Haematological disorders, glycemia, and disease processes can also increase or decrease the resistance of erythrocytes to haemolysis (Kobo et al., 2014). Nutrition as to the type and nature of feed can also affect the osmotic fragility of the erythrocytes (Aro et al., 2013; Aro, 2018).

The aim of this experiment is to investigate the whole blood viscosity and erythrocyte osmotic fragility of laying hens reared in battery and furnished cages.

2. Materials and Methods

2.1. Study Location

The study was conducted on a commercial Farm in Ado-Ekiti, Ekiti State, Nigeria (Latitude 7.6124°N and Longitude 5.2371°E). The location of farm is located in South Western Nigeria, where the rainy season lasts for about eight months (March to October) with about 1524mm of rainfall per annum and an atmospheric temperature of between 26 and 31°C, with a mean annual relative humidity of about 80%.

2.2. Experimental Birds

A total of 900 Isa Brown laying hens were purchased at day old from a reputable farm and reared from day old till 14 weeks in a brooding pen and growing pen. At 14 weeks, 790 birds were weighed and randomly divided into three treatment groups: large furnished cages (LFC), small furnished cages (SFC), and conventional battery cages (CBC), in a completely randomized design, with LFC having 10 replicates, and SFC and CBC having 15 and 18 replicate respectively. There were 40 hens in each LFC replicate, 8 hens in each SFC replicate, and 15 hens in each CBC replicate. The experiment started at the 14th week of age and ended at the 60th week of age.

2.3. Experimental Cages

Furnished cages were designed by a commercial cage manufacturer. For CBC, measurement was at a stocking density of 480cm^2 per bird, while the SFC and the LFC were at a stocking density of 1000cm^2 per bird.
The dimensions of the cages are presented below.

Parameters	LFC	SFC	CBC
Dimension of cage (length by width by height) (cm)	300 by 133 by 100	120 by 67 by 45	195 by 37 by 37
No. of hens	40	8	15
Stocking Density (hens/m^2)	10	10	22
Front cage height (cm)	114	52	40
Rear cage height (cm)	110	49	35
Floor area (cm^2)	40000	8000	7200
Average area (cm^2/bird)	1000	1000	480
Nest size (length by width by height) (cm)	50 by 50 by 35	40 by 50 by 27	
No. of nests	4	1	
Nest area (cm^2)	10000	2000	
Average nest area (cm^2/bird)	250	250	
Sandbox (length by width by height) (cm)	60 by 50 by 4	40 by 30 by 4	
No. of sandboxes	2	1	
Sandbox area (cm^2)	6000	1200	
Average sandbox area (cm^2/bird)	150	150	
Length of long perch (cm)	300	80	
Length of short perch (cm)	133		

Table 1: Detailed Measurements of Experimental Cages

2.4. Health, Management, and Feeding

All the hens were housed in the same building and received identical standard feeding and management. Birds were fed with chicks' mash from day old till the 8th week of age and thereafter with growers' mash till the 20th week of age. Layers' mash was given to birds from the 21st week till the end of the experiment. Standard commercial diets were used. Daily feeding and cleaning routine was carried out from 06:00 to 07.00 and 13.00 to 14.00 hours. Sawdust was distributed in the dust bath of the furnished cages once a week. All necessary medications and vaccinations were given from day old till the end of the experiment to keep the birds in good health.

Nutrients	Chicks' Mash	Growers' Mash	Layers' Mash
Metabolizable Energy kcal/kg	2940	2600	2450
Crude Protein %	21	15.5	16.5
Crude Fat %	7	6	5
Calcium %	1.18	2	3.6
Av. Phosphorus %	0.64	0.3	0.45
Lysine %	1.45	0.82	0.8
Methionine %	0.65	0.39	0.34

Table 2: Composition of Commercial Diets Used in the Experiment

2.5. Data Collection

Blood samples were collected in the 60th week. The blood sample was collected from 4 birds per replicate (LFC), 2 birds per replicate (SFC), and 2 birds per replicate (CBC). Cotton wool soaked in 70% alcohol was used to clean the surface of the site of blood collection, and thereafter, blood was collected from the jugular vein of each bird using a sterile needle and a syringe. 5 mls of blood was collected and transferred to plastic sample bottles containing ethylene diamine tetraacetic acid (EDTA) to prevent coagulation. Blood samples were kept in ice packs until they were transported for analysis.

2.5.1. Whole Blood Viscosity

The whole blood viscosity was determined with the use of an Oswald viscometer and a stopwatch. The values of the whole blood viscosity were calculated from the values obtained from the viscometer reading using the formula below:

$$\text{Viscosity} = \frac{\text{Flow time of sample} \times 1.0038}{\text{Flow time of water}}$$ (Aro, 2014)

Where 1.0038 is the viscosity of water at standard temperature and pressure, and the flow time of water is 2.85 seconds.

2.5.2. Erythrocyte Osmotic Stability

The osmotic stability was determined as previously described by Oyewale (1991) using NaCl and distilled water. Briefly, ten bottles were each filled with 100ml of distilled water, after which 0.00g to 0.90g of NaCl was measured and dissolved respectively into each of the bottles to give a saline concentration that ranged from 0.00% to 0.90%. A measured quantity (0.05 milliliter) of the blood collected from the birds was added to the saline solution in the test tubes from which the percentage of the red blood cells hemolyzed per saline concentration was calculated and used as a measure of red blood cell osmotic stability.

2.6. Statistical Analysis

All data were analyzed using the General Linear Model procedure of SAS version 9.3 (2011) for windows by SAS Institute Inc., Cary, NC, USA. Means were separated using Duncan Multiple Range Test, and significance was set at P-value ≤ 0.05.

3. Results and Discussion

Table 3 shows the whole blood viscosity (centiPoise) of laying hens reared in large furnished (LFC), small furnished (SFC), and conventional battery cages (CBC). It can be seen from the table that there was no significant difference in the blood viscosities of laying hens reared across the three cage types. This could be an indication that the stress levels of birds in the cages are similar.

Figure 1 shows the erythrocyte osmotic stability of red blood cells of laying hens reared in large furnished (LFC), small furnished (SFC), and conventional battery cages (CBC). The figure shows that CBC hens had the highest number of stable red blood cells, at 0.0 to 0.9% NaCl concentrations, compared to LFC and SFC hens. The result also showed that the minimum and maximum osmotic fragility for the laying hens in this study was found to be at 0.00 % and 0.90 % saline concentration, and a progressive decrease in red blood cells stability was noticed as the concentration of the salt decreased from 0.9 % to 0.00 %.

Erythrocyte Osmotic Stability Test (EOS) is used to measure erythrocyte resistance to hemolysis while being exposed to varying levels of dilution of a saline solution. It can be an indication of the adaptability of laying hens to a particular condition. CBC hens had the highest number of stable red blood cells compared to LFC and SFC hens, that is, birds raised in CBC were more osmotically stable than birds raised in LFC and SFC. This can be an indication that the birds adapt better to CBC than to LFC and SFC. The reduction in the number of stable red blood cells in LFC could be a result of

the larger group size in LFC that could lead to an increased body temperature of the birds in LFC as EOS has been found to be influenced by temperature (Oyewale et al., 2011).

Cage Type	Blood Viscosity
Lfc	2.53 ± 0.073
Sfc	2.44 ± 0.053
Cbc	2.44 ± 0.043
P-Value	0.38

Table 3: Whole Blood Viscosity (Centipoise) of Laying Hens Reared in Large Furnished, Small Furnished, and Conventional Battery Cages

- LFC= Large Furnished Cages,
- SFC= Small Furnished Cages,
- CBC= Conventional Battery Cages

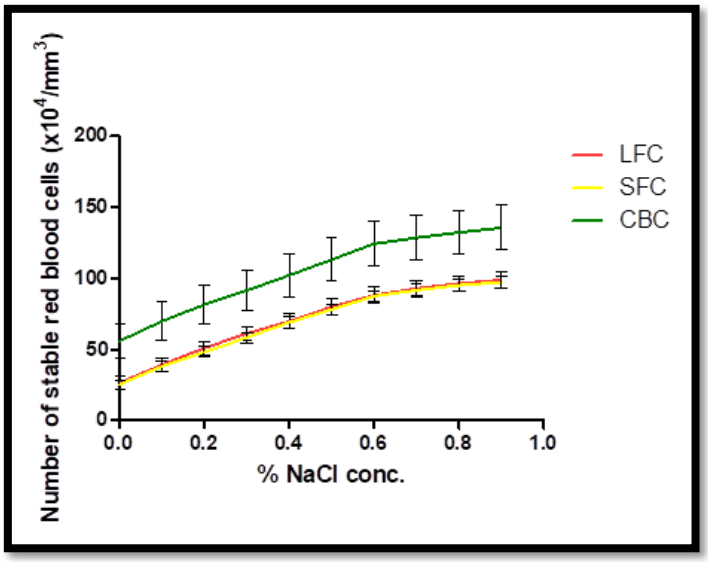

Figure 1: Number of Stable Red Blood Cells in Laying Hens Reared in Large Furnished Cages (LFC), Small Furnished Cages (SFC) and Conventional Battery Cages (CBC)

4. Conclusion

The blood viscosity of laying hens reared in battery, and furnished cages are similar. Also, laying hens are more osmotically stable in battery cages than in furnished cages.

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