Impact of management systems on selenium and zinc levels, heterophil: Lymphocyte ratio as biomarkers of immunity in chickens

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Abstract
This study investigated the impact of poultry management systems (intensive and extensive) on selenium and zinc concentrations and heterophil to lymphocyte ratio in chickens. Sixty chickens comprising 20 broilers, 20 local chickens and 20 layers obtained from poultry markets and houses around Ibadan, Oyo State, Nigeria were used in this study. Five millilitres of blood were collected through the brachial vein to analyse for selenium, zinc and complete blood count using atomic absorption spectrophotometry and standard haematological methods, respectively. There was a significantly (P<0.05) higher red blood cell, haemoglobin and pack cell volume values in local chickens raised in the extensive management system compared to broilers and layers raised in the intensive management system. There was no significant difference (P>0.05) in the white blood cell count between layers and local chickens. However, layers had higher white blood cell counts than local chickens. In contrast, local chickens had significantly (P<0.05) lower white blood cell count than broilers. The respective lymphocyte and heterophils counts were significantly (P<0.05) higher and lower in local chickens raised extensively compared to layers raised intensively. There were no significant differences (P>0.05) in monocyte, eosinophil and basophil count in chickens raised from both management systems. Chickens raised in the extensive management system had a significantly (P<0.05) higher selenium concentration with a lower heterophil to lymphocyte ratio than those raised in the intensive management system but had a significantly (P<0.05) lower zinc concentration. Therefore, high selenium levels and low heterophil to lymphocyte ratio could contribute to the resistance of chickens to various stressors associated with an extensive management system as selenium is a known immunomodulator.

Keywords: Chickens, Heterophil: Lymphocyte ratio, Management systems, Selenium, Zinc
Introduction

The Sustainable Development Goal (SDG-2) aimed at ending hunger, achieving food security, improving nutrition and promoting sustainable agriculture could be achieved through researches focusing on enhancing livestock nutrition (FAO, 2016). Also, the rapidly increasing human population in developing countries has necessitated the need for an increase in animal production especially poultry to meet the increasing animal protein source requirements (Ayele et al., 2009; Lawal et al., 2016). Management systems of chickens are crucial to influencing their health and production efficiency to meet and sustain the increasing demand of animal source proteins (Ovwigho et al., 2009; Abd El- Hack et al., 2019). There are several types of management systems in poultry productions: Organic, free range, cage and deep litter. They are generally classified as extensive, semi-intensive and intensive rearing system with each having its benefits and limitations (Izunobi, 2002; Mangesha, 2012; Elson, 2015). The cost of feeding in the extensive system is cheap as the birds feed from the surrounding vegetation with a better welfare but it requires more floor space, and the birds raised in this system are more susceptible to predators, unfavourable weather conditions amongst other shortcomings (Hayat et al., 2014; Ghanima et al., 2020). An intensive rearing system is more expensive, requiring high investment in cage installation and feeding, including the ease of disease transmission. However, it provides the convenience of cleaning and disinfection of pens, efficient record-keeping and prompt culling of sick and unproductive birds (Addass et al., 2012; Shields & Greger, 2013). The semi-intensive system is reported to minimise the limitations of both intensive and extensive rearing systems by allowing birds to source for their feed alongside supplementing their feed and also providing them with shelter (Mangesha, 2012; Wantasen et al., 2014).

Nutrition is required for the maintenance of normal homeostatic condition, growth, reproduction and immune responses. Micronutrients are an important component of nutrition and are essential for these physiologic processes associated with immunity (Surai, 2002; Calder et al., 2020). Consequently, providing adequate amounts of micronutrients to meet animal requirements is critical to maximising animal health and productivity (Suttle, 2010; Aksu et al., 2012). Several researchers suggest that infection and other disease conditions can further deplete the body’s supply of these minerals, thus, leading to immunosuppression, reproductive disorders and alteration of several metabolic processes (Eijkelkamp et al., 2019; Read et al., 2019). This deficiency causes increased susceptibility to diseases by exposing cells to oxidative stress causing damage to cells and tissues from free radical species thus affecting immunity. Micronutrients and vitamins including zinc, selenium, iron, copper, manganese, vitamins A, C, E, and B6, folic acid, are important antioxidants known to have positive influences on growth, production and immune responses (Dizdar et al., 2016). Concentrations of micronutrients can be evaluated from blood analysis as it could help in specific diagnosis of some poultry pathologies and serve as a fundamental basis for studying avian micronutrient levels in health and disease (Bonadiman, 2009). Also, several physiological and environmental factors that might affect blood values such as the effects of housing, feeding, breeding method and animal breed have been identified (Bashar et al., 2010).

Chickens raised under extensive systems are able to roam around freely and express their natural behaviours which include selecting their feed from various plants, herbs and insects containing varying concentrations of micronutrients (Ovwigho et al., 2009). This is however absent in the intensive rearing systems as birds are provided feed in which the levels of micronutrients in the plants used in the feed production depend on several factors such as types of grain, the season of harvest and climate change (Moravej et al., 2012). Poultry having an optimal amount of minerals, especially micronutrients, can improve their body metabolism, ensure normal functioning of the immune system, and enhance their natural resistance to disease. Studies on haematological parameters give a good understanding of the nutritional status of the feed fed to poultry. They also provide useful information on the metabolic profile, which is used to assess the bird’s state of health (Wayas et al., 2018). Evaluation of blood parameters during haematological studies in avian medicine is important in disease diagnosis, determination of immunologic status and has a positive correlation with nutrition (Adeyemo et al., 2018). According to Nyalulingo (2013), there was no significant effect of management systems on haematological parameters, while other researchers have reported variation on the impact of different management systems on haematological and biochemical parameters of chickens (Saurabh et al., 2018; Goyal et al., 2018). In addition, there are reports on the importance of poultry feed supplementation with micronutrients because their...
deficiency is associated with the production of reactive oxygen species which exposes cells especially erythrocytes to oxidative stress (Zheng et al., 2019). Also, other researchers (Chen et al., 2014; Okunola et al., 2015; Fawzy et al., 2016) have reported the influence of micronutrient supplementation in poultry feed by evaluating immunologic response, growth, production and stress. Poultry is exposed to various forms of stress, including transportation, debeaking, vaccination, overcrowding, temperature changes and diseases (Quinteiro-Filho et al., 2012; Zhao et al., 2014). Exposure of chickens to these forms of stresses stimulates the release of corticosterone from the adrenal gland into the blood circulation resulting in increased circulation of heterophil with an associated lymphopaenia (Clark, 2015). Thus, heterophil/lymphocyte ratio is being reported to be a biomarker of stress and a disease resistance trait (Scanes et al., 2016). However, there is paucity of information regarding the impact of different management systems on micronutrient concentrations in different types of chicken, with very little recent data comparing the impact of these systems on the relationship between micronutrients and heterophil: lymphocyte ratio as a biomarker of immunity and resistance trait in chickens. The need, therefore, arises to investigate the impact of different poultry management systems on biomarkers of immunity in chickens raised in Ibadan, Oyo State, Nigeria.

Materials and Methods

Ethics approval
The University of Ibadan Animal Care and Use Research Ethics Committee guidelines were followed for this study with approval number UI-ACUREC/20/042.

Chickens and management systems
Sixty chickens comprising of 20 broilers, 20 local chickens and 20 layers obtained from poultry markets and houses around Ibadan, Oyo State, Nigeria were used in this study. The broilers and layers were reared using the cage system (intensive system) while the local chickens were raised on a free-range system (extensive system).

Blood sample collection
Five millilitres of blood sample were collected from each bird on every sampling day through the brachial vein for selenium, zinc, and complete blood count analysis, using atomic absorption spectrophotometry and standard haematology procedures respectively. Serum selenium and zinc analysis: Three millilitres of the blood samples from each bird were collected into sterile test tubes and allowed for 30 minutes to clot. The clotted samples were centrifuged at 3000 rpm for 10 minutes. Serum samples were harvested and used immediately for the determination of serum selenium and zinc levels using atomic absorption spectrophotometry as described by Al-Assaf (2010).

Complete blood count analysis: Two millilitres of the blood samples from each bird were collected into a properly labelled heparinised sample bottle and used immediately for haematological analysis using standard procedures. Packed cell volume (PCV) was determined by the microhaematocrit method (Benson et al., 1989), while red blood cell (RBC) and white blood cell (WBC) counts were done by the haemocytometer method using Natt and Herrick solution as the diluting fluid (Lamb et al., 1981). Haemoglobin concentration was determined by the cyaemthaemoglobin method (Jain, 1986) while smears for thrombocyte count and differential WBC count (neutrophils, eosinophils, basophils, lymphocytes and monocytes) for each blood sample were prepared and stained by the Giemsa technique and enumerated (Tavares-Dias & Moraes, 2007).

Statistical analysis
All data obtained from this experiment were statistically analysed using the Student t-test and one-way analysis of variance (ANOVA). The relevance of the differences was estimated by applying Duncan’s multiple range tests. SPSS version 20 software package was used for analysis, and values with P<0.05 were considered statistically significant.

Results
The PCV values of local chickens reared under the extensive management system (EMS) were significantly (P < 0.05) higher than those of layers reared under the intensive management system (IMS). However, there was no significant difference (P>0.05) between (EMS) local chickens and (IMS) broilers (Table 1), while within the IMS the PCV values of broilers were significantly (P< 0.05) higher than those of the layers. The haemoglobin and red blood cell values of the local chickens (EMS) were significantly (P < 0.05) higher than those of (IMS) broiler and layer chickens (Table 1). In IMS group, the haemoglobin and RBC values of broilers were significantly (P< 0.05) higher
than those of the layers. The thrombocyte value was significantly (P<0.05) higher in the (IMS) broiler than in the layers and local chickens.

The total WBC values of local chickens reared under the EMS were significantly (P < 0.05) lower than those of broilers reared under IMS (Table 2). However, there was no significant difference (P > 0.05) between EMS local chickens and IMS broilers, while within the IMS the total WBC values of broilers were significantly (P < 0.05) higher than those of the layers (Table 2). The lymphocyte counts of local chickens reared under the EMS were significantly (P < 0.05) higher than those of layers reared under IMS but significantly (P < 0.05) lower than those of broilers reared under the IMS (Table 2). However, there was no significant difference (P > 0.05) between EMS local chickens and IMS broilers, while within the IMS the lymphocyte values of broilers were significantly (P < 0.05) higher than those of the layers (Table 2). The heterophil counts of local chickens reared under the EMS were significantly (P < 0.05) lower than those of layers reared under IMS (Table 2). However, there was no significant difference (P > 0.05) between EMS local chickens and IMS broilers, while within the IMS the heterophil counts of broilers were significantly (P < 0.05) lower than those of the layers (Table 2). The monocyte, eosinophil and basophil counts of EMS reared local chickens showed no significant (P > 0.05) differences when compared with those of IMS reared broiler and layer chickens (Table 2).

The serum selenium levels of local chickens reared under the EMS were significantly (P < 0.05) higher than those of broilers and layers reared under IMS (Table 3). There was no significant difference (P > 0.05) between the IMS reared broiler and layer chickens (Table 3). The serum zinc levels of local chickens reared under the EMS were significantly (P < 0.05) lower than those of broilers and layers reared under the IMS (Table 3). There was no significant difference (P > 0.05) between the serum zinc levels of IMS reared broiler and layer chickens (Table 3).

**Table 1**: Mean± standard deviation of erythrogram parameters of layers, broilers and local chickens reared using the different management systems

<table>
<thead>
<tr>
<th>Variables</th>
<th>Management system</th>
<th>Layers (n=20)</th>
<th>Broilers (n=20)</th>
<th>Local Chicken (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>Intensive</td>
<td>26.05±3.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.70±3.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.30±5.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>Extensive</td>
<td>8.44±1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.62±1.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.69±1.54&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>RBC (x10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>Intensive</td>
<td>2.51±0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.06±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.44±0.41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PLATELET (x10&lt;sup&gt;9&lt;/sup&gt;)</td>
<td>Intensive</td>
<td>1.39±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.66±0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.60±0.60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

PCV= Packed Cell Volume, Hb= Haemoglobin concentration, RBC= Red Blood Cell.

<sup>a,b,c</sup> Means in the same row with different superscripts are significantly different (P<0.05)

**Table 2**: Mean± standard deviation of leukogram parameters of layers, broilers and local chickens reared using the different management systems

<table>
<thead>
<tr>
<th>Variables</th>
<th>Management system</th>
<th>Layers (n=20)</th>
<th>Broilers (n=20)</th>
<th>Local Chickens (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x10&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>Intensive</td>
<td>14.10±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.50±0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.80±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>Extensive</td>
<td>59.65±4.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.00±5.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.25±5.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heterophil (%)</td>
<td>Intensive</td>
<td>33.30±4.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.80±5.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.65±4.56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>Extensive</td>
<td>3.40±0.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.05±0.95&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.70±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>Intensive</td>
<td>3.50±1.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.65±1.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.15±1.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>Extensive</td>
<td>0.20±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30±0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

WBC= White Blood Cell. <sup>a,b,c</sup> Means in the same row with different superscripts are significantly different (P<0.05)

**Table 3**: Mean± standard deviation of micronutrient concentrations of layers, broilers, and local chickens reared using the different management systems

<table>
<thead>
<tr>
<th>Variables</th>
<th>Management system</th>
<th>Layers (n=20)</th>
<th>Broilers (n=20)</th>
<th>Local Chickens (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium (µg/l)</td>
<td>Intensive</td>
<td>0.11±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78±0.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc (µg/l)</td>
<td>Extensive</td>
<td>1.25±1.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00±1.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means in the same row with different superscripts are significantly different (P<0.05)
Table 4: Mean± standard deviation of heterophil, lymphocyte and heterophil: lymphocyte (H: L) ratio of chickens reared using different management systems

<table>
<thead>
<tr>
<th>Variables</th>
<th>Heterophil</th>
<th>Lymphocyte</th>
<th>H: L Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensive system</td>
<td>33.30±4.24a</td>
<td>29.80±5.40a</td>
<td>0.57±0.12a</td>
</tr>
<tr>
<td>Extensive system</td>
<td>29.65±4.56b</td>
<td>63.25±5.0b</td>
<td>0.47±0.11b</td>
</tr>
</tbody>
</table>

a,b. Means in the same column with different superscripts are significantly different (P<0.05)

There was a significantly (P<0.05) higher heterophil value in the IMS compared to the EMS, while the lymphocyte value in the IMS was significantly (P<0.05) lower compared to the EMS (Table 4). Also, the intensive management system had a significantly (P<0.05) higher H: L ratio than the extensive management system (Table 4). In addition, the EMS had a significantly (P<0.05) higher selenium concentration with a lower H: L ratio when compared with the intensive management system having a lower selenium concentration and a higher H: L ratio (Figure 1).

Discussion

The PCV of both layers and broilers were within the normal reference range (22-35 %) as reported by Jain (1986). This could be associated with the type of management system as chickens reared intensively, especially for commercial purposes, are provided with feed and water ad libitum (Boostani et al., 2010; Etim et al., 2014). In the EMS, local chickens had a significant (P≤0.05) higher PCV compared to chickens in the IMS. This was, though, lower than the reference interval (35.90-41.00%) as proposed by Mitruka & Rawnsley (1997) for local chickens. This finding agrees with the reports by Solomon & Udoh (2017) as they associated anaemia in local chickens in their study with the scarce availability of feed usually common with the extensive system of management and also their genotypic differences.

The EMS local chickens had significantly (P < 0.05) higher PCV and haemoglobin values than the IMS broilers and layers. The Hb values in these chickens correspond with their PCV values as chickens with higher PCV had higher haemoglobin concentrations. These findings are in line with the report by Naglaa & Abozaid (2018), who also observed a corresponding higher haemoglobin concentration in migratory birds with higher PCV. A contrasting report was observed from the findings of Iheukwumere et al. (2006), who reported lower Hb concentration; this could be associated with specie difference as broilers were the study animals. The size, haemoglobin content, and the average concentration of circulating erythrocytes are useful indicators for RBC indices (Glomski and Pica, 2011). The RBC concentration in chickens reared in the EMS also had a significant (P<0.05) higher value as it was observed for the haemoglobin concentration compared to chickens reared in the IMS.

The WBC are defence cells of the body that help to fight against infection and diseases (Egu, 2017; Naglaa & Abozaid, 2018). In poultry, varying levels of circulating WBC counts, especially lymphopenia and heterophilia, are good indicators of stress (Bin-Jumah et al., 2020). Broilers had a significantly (P<0.05) higher WBC count compared to layers and local chickens. Chickens with higher WBC count could have the ability to withstand the varying levels of stress chickens are exposed to in various management systems (Adeyemo et al., 2018). The findings from this study disagree with the report from Iheukwumere et al. (2006), who reported lower WBC values in Nigerian indigenous chickens. However, this finding is in line with the findings by Saurabh et al. (2018), who reported no difference in total WBC counts of chickens reared under different management systems. The lymphocyte and heterophil values in
both management systems were within the reference intervals (47.2 to 85.0% and 10.00 to 53.60%), respectively as reported by Riddell (2011). It could be inferred that different management systems do not affect their concentrations.

The eosinophil and monocyte counts in this study for all the chickens were within the normal reference range (0.00 to 15.00% and 0.00 to 5.67%, respectively) as proposed by Riddell (2011). Also, the basophil count was between the normal reference ranges (0.00 to 3.33%) and agrees with findings by Olukomaiya et al. (2014). The basophil value in haematological studies indicates varying levels of histamines in circulating blood (Afolabi et al., 2011). Besides environmental and physiologic factors, nutrition as well as management system are reported to cause variations in not only haematology indices but also micronutrient levels in chickens (Nyaulinga, 2013; Oluwadiya et al., 2017). In this study, chickens reared under the different systems of management had micronutrients concentrations (selenium and zinc) similar to those reported by Kinal et al. (2012). Although layers had higher micronutrient concentrations compared to broilers, it was not statistically significant (P>0.05). The higher values in layers could be associated with the availability of feed for a longer period of time as they are kept commercially for egg production purposes as opposed to broilers which are raised for meat before being processed for commercial uses (Gajana et al., 2011). A contrary result was obtained when the two types of management systems of rearing chickens were compared. There was a significantly (P<0.05) higher micronutrient concentration (selenium) in chickens reared using the extensive system when compared with the intensive system. This could be associated with the ability of the chickens to express their natural behaviour through better foraging activity, enabling accessibility of chickens to various source of feed including pasture, earthworm, insect, soil, food waste and by-products which have varying concentrations of these micronutrients (Hayat et al., 2014). This process is absent in birds raised in the intensive system as they are only fed commercial feed (Gajana et al., 2011).

Higher micronutrient levels in local chickens for selenium could be responsible for their ability to withstand and survive the array of environmental stress factors associated with the extensive system of rearing, resulting in their better adaptation to the rearing conditions (Sirri et al., 2010). In chickens, a high H: L ratio has been associated with increased stress and less resistance to disease, while a low H: L ratio implies less stress and increased resistance to diseases (Scanes, 2016). This finding is in line with reports by Scholz et al. (2008) and Shimmura et al. (2010), who also reported a lower H: L ratio in chickens reared under the free-range or alternative poultry housing systems.

In this study, chickens raised extensively had higher (P<0.05) selenium concentration with a low H: L ratio compared with layers reared intensively, having lower selenium concentration and higher H: L ratio. This could be associated with the role of selenium as an antioxidant responsible for mitigating stress and improving the immune status of chickens (Gao et al., 2012). Therefore, the low H: L ratio in local chickens suggests the presence of resistance trait in them and could be associated with the high levels of selenium (an active immunomodulatory); thus, their ability to withstand the mirage of harsh environmental conditions associated with the extensive system of poultry production.

In conclusion, chickens raised under the extensive system of management had higher selenium concentration with an associated low heterophil to lymphocyte ratio compared to those raised under the intensive management system. This is suggestive of the presence of resistance traits in local chickens to various forms of stressors possibly associated with the role of selenium as an active immunomodulator and antioxidant. This preliminary study serves as a source of baseline data for selenium and zinc concentration in chickens raised using both intensive and extensive management systems in Ibadan, Oyo State, Nigeria.

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Conflict of interest
The authors declare that there is no conflict of interest.

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