RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v20i5.13

Adekunle *et al.*/*Sokoto Journal of Veterinary Sciences*, **20**(Special): 114 – 122.

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

LA Adekunle[1], SY Idris[2], AE Sijuwola[1], AJ Jubril[1]* & VO Taiwo[1]

1. *Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria*
2. *Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

*Correspondence: Tel.: +2348034701005; E-mail: afusatjagun@gmail.com

Publication History:
Received: 28-12-2021
Revised: 15-04-2022
Accepted: 16-04-2022

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 heterophil 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 Nyaulingo (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 cyanmethaemoglobin 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 layers, 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

Management system	Intensive		Extensive
Variables	Layers (n=20)	Broilers (n=20)	Local Chicken (n=20)
PCV (%)	26.05±3.47^{a}	29.70±3.81^{b}	31.30±5.10^{b}
Hb (g/dl)	8.44±1.08^{a}	9.62±1.22^{b}	10.69±1.54^{c}
RBC (x10^{6})	2.51±0.79^{a}	3.06±0.32^{b}	3.44±0.41^{c}
PLATELET (x10^{9})	1.39±0.22^{a}	2.66±0.49^{b}	1.60±0.60^{c}

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

^{a,b,c} 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

Management systems	Intensive		Extensive
Variables	Layers (n=20)	Broilers (n=20)	Local Chickens (n=20)
WBC (x10^{3})	14.10±0.13^{a}	16.50±0.36^{b}	14.80±0.21^{a}
Lymphocyte (%)	59.65±4.59^{a}	64.00±5.80^{c}	63.25±5.0^{c}
Heterophil (%)	33.30±4.24^{a}	29.80±5.40^{b}	29.65±4.56^{b}
Monocyte (%)	3.40±0.99^{a}	3.05±0.95^{ab}	2.70±0.73^{a}
Eosinophil (%)	3.50±1.50^{a}	3.65±1.35^{a}	4.15±1.57^{a}
Basophil (%)	0.20±0.41^{a}	0.30±0.47^{a}	0.25±0.44^{a}

WBC= White Blood Cell. ^{a,b,c} 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

Management system	Intensive		Extensive
Variables	Layers (n=20)	Broilers (n=20)	Local Chickens (n=20)
Selenium (µg/l)	0.11±0.13^{a}	0.07±0.03^{a}	0.78±0.61^{b}
Zinc (µg/l)	1.25±1.50^{a}	1.00±1.12^{a}	0.25±0.25^{b}

^{a,b,} 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

Variables	Heterophil	Lymphocyte	H: L Ratio
Intensive system	33.30±4.24[a]	29.80± 5.40[a]	0.57±0.12[a]
Extensive system	29.65±4.56[b]	63.25±5.0[b]	0.47±0.11[b]

[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).

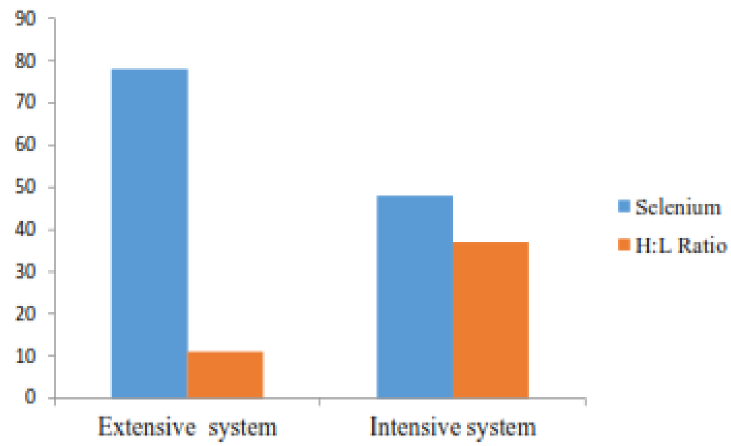

Figure 1: The relationship between selenium and H: L ratio comparing the extensive and intensive system of management in chickens

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 to15.00% 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 (Nyaulingo, 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.

Acknowledgements

The authors appreciate the support of Mrs J Ademakinwa and Mrs FA Adeyeyi of the Veterinary Clinical Pathology Laboratory, University of Ibadan, for their role in the analysis of samples used in the study.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Abd El-Hac ME, Alagawany M & Noreldin AE (2019). Managerial and Nutritional Trends to Mitigate Heat Stress Risks in Poultry Farms. In: *Sustainability of Agricultural Environment in Egypt*: Part II. Springer, Cham. Pp 325–338.

Addass PA, David DL, Edward A, Zira KE & Midau A (2012). Effect of age, sex and management system on some haematological parameters of intensively and semi-intensively kept chicken in Mubi, Adamawa State, Nigeria. *Iranian Journal of Applied Animal Science*, **2**(3): 277-282.

Adeyemo GO, Bolarinwa MO & Ehiabhi O (2018). Body image, social anxiety and psychological distress among young adults. *International Journal of Molecular Biology,* **3**(4): 197–201.

Afolabi KD, Akinsoyinu AO, Abdullah ARO, Olajide R & Akinleye SB (2011). Haematological parameters of the Nigerian local grower chickens fed varying dietary levels of palm kernel cake. *Poljoprivreda,* **17**(1): 74-78.

Al-Assaf NA (2010). Determination of serum trace elements magnesium, copper, zinc, and selenium in asthmatic patients by atomic absorption spectrophotometry. *Al-Nahrain Journal of Science*, **13**(1): 20-25.

Aksu DS, Aksu T & Önel SE (2012). Does inclusion at low levels of organically complexed minerals versus inorganic forms create a weakness in performance or antioxidant defence system in broiler diets? *International Journal of Poultry Science,* **11**(10): 666-672.

Ayele G, Asare-Marfo D, Birol E & Roy D (2009). Investigating the role of poultry in livelihoods and the impact of HPAI in Ethiopia. Controlling Avian Flu and protecting people's livelihoods in Africa and Indonesia. International Food Policy Research Institute (IFPRI) with the International Livestock Research Institute (ILRI) and Royal Veterinary College (RVC). Pp 12.

Bashar A, Ahmed R, Ahmed I, Jahan R, Ahsan S & Chowdhury H (2010). A survey on the use of medicinal plants by folk medicinal practitioners in three areas of Pirojpur district, Bangladesh. *American- Eurasian Journal of Sustainable Agriculture*, **4**(2): 247-259.

Benson HJ, Gunstream SE, Talaro A & Talaro KP (1989). *Anatomy and Physiology Laboratory Texbook*. Win. C. Brown Publisher Dubuque Iowa, USA. Pp 1- 672.

Bin-Jumah M, Abd El-Hack ME, Abdelnour SA, Hendy YA, Ghanem HA, Noreldin AE, Khafaga AF, Alsafy SA, Shaheen H, Samak D, Momenah MA, Allam AA, Aleya L & Abdel-Daim MM (2020). The potentiality of using chromium as an attenuator agent of thermal stress in animals: A review. *Science of the Total Environment*, doi.10.1016/j.scitotenv.2019.135996.

Bonadiman SF, Stratievsky GC, Machado, JA, Albernaz AP, Rabelo GR & Damatta, RA (2009). Leukocyte ultrastructure, haematological and serum biochemical profiles of ostriches (*Struthio camelus*). *Poultry Science*, **88**(11): 2298-2306.

Boostani A, Ashayerizadeh A, Mahmoodian FHR & Kamalzadeh A (2010). Comparison of the effects of several feed restriction periods to control ascites on performance, carcass characteristics and haematological indices of broiler chickens. *Revista Brasileira de Ciência Avícola,* **12**(3): 170– 177.

Calder PC, Carr AC, Gombart AF & Eggersdorfer M (2020). Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients*, **d**oi.10.3390/nu12041181.

Chen G, Wu J & Li C (2014). Effect of different selenium sources on production performance and biochemical parameters of broilers. *Journal of Animal Physiology and Animal Nutrition*, **98**(4): 747-754.

Clark P (2015). Observed variation in the heterophil to lymphocyte ratio values of birds undergoing investigation of health status. *Comparative Clinical Pathology*, **24**(5): 1151–1157.

Dizdar OS, Baspınar O, Kocer D, Dursun ZB, Avci D, Karakükcü C, Çelik İ & Gundogan K (2016). Nutritional risk, micronutrient status and clinical outcomes: A prospective observational study in an infectious disease clinic. *Nutrients*, **8**(3): 124.

Egu UN (2017). Haematological and serum biochemical parameters of mature harco cocks treated with human menopausal gonadotrophin (diclair®) for spermatogenesis. *International Journal of Environment, Agriculture and Biotechnology*, **2**(1): 429-436.

Eijkelkamp BA, Morey JR, Neville SL, Tan A, Pederick VG, Cole N, Singh PP, Ong CY, Gonzalez de Vega R, Clases D, Cunningham BA, Hughes CE, Comerford I, Brazel EB, Whittall JJ, Plumptre CD, McColl SR, Paton JC, McEwan AG, Doble PA & McDevitt CA (2019). Dietary zinc and the control of *Streptococcus pneumoniae* infection. *PLoS Pathogen*, doi.10.1371/journal.ppat.1007957.

Elson HA (2015). Poultry welfare in intensive and extensive production systems. *World's Poultry Science Journal*, **71**(3): 449-460.

Etim NN, Williams ME, Akpabio U & Offiong EEA (2014). Haematological parameters and factors affecting their values. *Agricultural Science*, **2**(1): 37-47.

FAO (2016). Food and agricultural organization: United Nations Decade of Action on Nutrition 2016–2025. http://www.fao.org/3/a-i6130e.pdf, retrieved 07-01-2020.

Fawzy MM, El-sadawi HA & El-dien MH (2016). Haematological and biochemical performance of poultry following zinc oxide and sodium selenite supplementation as food additives. *Annals of Clinical Pathology,* **4**(4): 1076-1085.

Gajana CS, Nkukwana TT, Chimonyo M & Muchenje V (2011). Effect of altering the starter and finisher dietary phases on growth performance of broilers. *African Journal of Biotechnology*, **10**(64): 14203-14208.

Gao X, Xing H, Li S, Li J, Ying T and Xu S (2012). Selenium regulates gene expression of selenoprotein W in chicken gastrointestinal tract. *Biological Trace Element Research*, **145**(2): 181–188.

Ghanima MA, Abd El-Hack ME, Othman SI, Taha AE, Allam AA & Eid Abdel-Monei AM (2020). Impact of different rearing systems on growth, carcass traits, oxidative stress biomarkers, and humoral immunity of broilers exposed to heat stress. *Poultry Science,* **99**(6): 3070–3078.

Glomski CA & Pica A (2011). *The Avian Erythrocyte: Its Phylogenetic Odyssey*. (NH Enfiels, editor): Science Publishers. USA. Pp 1-640.

Goyal G, Baghel KKS, Mishra AK, Narwaria US, Singh A K, Mandal S & Thakur R (2018). Effect of different rearing systems on heamato-biochemical parameters of Kadaknath chicken. *Journal of Animal Research*, **8**(6), 1091-1097.

Hayat A, Basheer A, Zahoor I & Mahmud A (2014). Free-range rearing system and its impact on production and consumption of poultry: A review. *Science International*, **26**(3): 1297-1300.

Iheukwumere FC, Abu AH & Ameh M (2006). Effect of human menopausal gonadotrophin on haematological and serum biochemical parameters of Nigerian indigenous chickens. *International Journal of Poultry Science*, **5** (7): 632-634.

Izunobi ND (2002). *Poultry Husbandry: An Integrated Approach for Tertiary Students Extension Agents Policymakers and Farmers*. Ihiala NADS publishers, Nigeria. Pp 63-70.

Jain NC (1986). *Schalm's Veterinary Haematology.* Fourth edition, Lea and Febiger, USA, Pp 1221-1229

Kinal S, Król B & Tronina W (2012). Effect of various selenium sources on selenium bioavailability, chicken growth performance, carcass characteristics and meat composition of broiler chickens. *Electronic Journal of Polish Agricultural University*, **15**(1): 7-15.

Lamb RA, Lai CJ & Choppin PW (1981). Sequences of mRNAs derived from genome RNA segment 7 of influenza virus: Colinear and interrupted mRNAs code for overlapping proteins. *Proceedings of the National Academy of Sciences*, **78**(7): 4170-4174.

Lawal JR, Jajere SM, Ibrahim UI, Geidam YA, Gulani IA, Musa G & Ibekwe BU (2016). Prevalence of coccidiosis among village and exotic breeds of chickens in Maiduguri, Nigeria. *Veterinary World*, **9**(6): 653–659.

Mangesha M (2012). Native chicken production and the innate characteristics. *Poultry Science*, **6**(2): 56-64.

Mitruka BM & Rawnsley HM (1997). *Clinical, Biochemical and Haematological Reference Values in Normal Experimental Animals.* Masson Publishing, USA. Inc. New York, Pp. 83-109.

Moravej H, Alahyari-Shahrasb M, Baghani M & Shivazad M (2012). Withdrawal or reduction of the dietary vitamin premix on bone parameters of broiler chickens in two rearing systems. *South African Journal of Animal Science*, **42**(2):169–177.

Naglaa AA & Abozaid ER (2018). Effect of human chorionic gonadotropin on gastric emptying in female rats: Is it a direct effect or by induced hyperthyroidism? *The Medical Journal of Cairo University*, **86**(6): 3047-3053.

Nyaulingo JM (2013). Effect of Different Management Systems on Haematological Parameters in Layer Chicken. MSc. Dissertation, Department of Animal Science and Production, Sokoine University of Agriculture, Morogoro, Tanzania. Pp 29-35.

Okunlola D, Akande TO & Nuga H (2015). Haematological and serum characteristics of broiler birds fed diets supplemented with varying levels of selenium powder. *Journal of Biology*, **5**(1):107–111.

Olukomaiya OO, Adeyemi OA, Sogunle OM, Abiola MO, Iwuchukwu PO and Emuveyan UP (2014). Effects of feed restriction and ascorbic acid supplementation on haematological parameters of marshall broiler chickens. *Indian Journal of Innovations and Development*, **3**(2): 18-22.

Oluwadiya BO, Adeyemi OA, Olukomaiya OO, Obasa OA, Bello KO & Ladokun AO (2017). Blood biochemical parameters and bacterial load in the gastrointestinal tract of broiler chickens. *Tropical Agriculture*, **94** (4): 382-395.

Ovwigho BO, Bratte L & Isikwenu JO (2009). Chicken management systems and egg production in Delta State, Nigeria. *International Journal of Poultry Science*, **8** (1): 21-24.

Quinteiro-Filho WM, Rodrigues MV, Ribeiro A, Ferraz-de-Paula V & Pinheiro ML (2012). Acute heat stress impairs performance parameters and induces mild intestinal enteritis in broiler chickens: Role of acute hypothalamic-pituitary-adrenal axis activation. *Journal of Animal Science*, **90**(6): 1986-1994.

Read SA, Obeid S, Ahlenstie C & Ahand LG (2019). The role of zinc in antiviral immunity. *Advances in Nutrition,* **10**(4): 696-710.

Riddell C. (2011). *Comparative Anatomy, Histology and Physiology of the Chicken*. Department of Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Pp 37.

Saurabh GG, Krishna KSB, Anjani KM, Upendra SN, Anil, KS, Sanju M, Pankaj KB & Rajat T (2018). Effect of different rearing systems on heamato-biochemical parameters of kadaknath chicken. *Journal of Animal Research*, **8**(6): 1091-1097.

Scanes CG (2016). Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. *Poultry Science*, **95**(9): 2208–2215.

Scholz B, Rönchen S, Hamann H, Pend H & Dist O (2008). Effect of housing system, group size and perch position on H/L ratio in laying hens. *European Poultry Science*, **72**(8): 174–180.

Shields S & Greger M (2013). Animal welfare and food safety aspects of confining broiler chickens to cages. *Animals*, **3**(2): 386-400.

Shimmura T, Hirahara S, Azuma T, Suzuki T, Eguchi Y, Uetake K & Tanaka T (2010). Multi-factorial investigation of various housing systems for laying hens. *British Poultry Science*, **51**(1): 31–42.

Sirri F, Castellini C, Roncarati A, Franchini A & Meluzzi A (2010). Effect of feeding and genotype on the lipid profile of organic chicken meat. *European Journal of Lipid Science and Technology*, **112**(9): 994–1002.

Solomon IP & Udoh UH (2017). Haematological indices in three genotypes (Naked Neck, Frizzled Feather, Normal Feathered) of Nigerian local chicken. *Advances in Life Science and Technology*, **54**(1): 29-34.

Surai PF (2002). Selenium in poultry nutrition I. Reproduction, egg and meat quality and practical applications. *World's Poultry Science Journal*, 58(4): 431-450.

Suttle N (2010). *Mineral Nutrition of Livestoc*k. Fourth edition. Commonwealth Agricultural Bureaux International, Oxfordshire, Uk. Pp 211-237.

Tavares-Dias M & Moraes FRD (2007). Leukocyte and thrombocyte reference values for channel catfish (Ictalurus punctatus Raf), with an assessment of morphologic, cytochemical, and ultrastructural features. *Veterinary Clinical Pathology*, **36**(1): 49-54.

Wantasen E, Elly FH & Santa NM (2014). The analysis of semi-intensive native chicken farming in rural communities. *Journal of the Indonesian Tropical Animal Agriculture*, **39**(2): 126-133.

Wayas FO, Kolanisi U & Chimonyo M (2018). Haematological and serum biochemical responses of ovambo chickens fed provitamin A biofortified maise. *Brazilian Journal of Poultry Science*, **20**(3):425-434.

Zhao ZG, Li JH, Li X & Bao J (2014). Effect of housing systems on behaviour, performance and welfare of fast-growing broilers. *Asian Australian Journal of Animal Science*, **27**(1): 140-146.

Zheng S, Zhao J, Xing H & Xu S (2019). Oxidative stress, inflammation, and glycometabolism disorder-induced erythrocyte haemolysis in selenium deficient exudative diathesis broilers. *Journal of Cell Physiology*, **234**(9): 16328–16337.