

## Evaluations of the effect of genetic variations on serum renal function markers, bilirubin and lipid profile of laying domestic fowls

<sup>1</sup>Nweze E.C, <sup>1</sup>Ukwani I.A, <sup>2</sup>Agbo C.C, <sup>1</sup>Ukwueze I.J & <sup>1</sup>Odo B.C

<sup>1</sup>Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Abia State,

<sup>2</sup>Department of Agricultural Engineering, Nnamdi Azikiwe University Awka, Anambra State, Nigeria

\*Corresponding author: [ernest.nweze@yahoo.com](mailto:ernest.nweze@yahoo.com); Tel: +2348066336466

### ABSTRACT

A total of 16, 18 weeks old chickens with mean weight  $1.99 \pm 0.12$  kg (1.85-2.30kg) were used to compare the serum biochemical characteristics of four chicken genotypes (breeds/strains) from south-eastern part of Nigeria. The chickens were grouped based on their genotypes (breeds/strain) in a Completely Randomized Design for the four-week study. The genotypes (breeds) considered were Black Australorp (BA), Isa Brown (IB), Noiler (NOI) and Local chicken (LC) at 4 chickens per genotype. At the end of the experiment, four (4) birds per genotype were drawn and their blood samples analysed for serum biochemical studies. The mean total cholesterol (TC), triacylglycerol (TAG) and very low density lipoprotein cholesterol (VLDL-C) concentrations of local chicken (LC) were significantly ( $P < 0.05$ ) higher than the Isa brown (IB) values. Similarly, the mean blood urea nitrogen (BUN) concentrations of LC was significantly ( $P < 0.05$ ) higher than the Noiler value. The mean high density lipoprotein cholesterol (HDL-C) of black Australorp (BA) was significantly ( $P < 0.05$ ) higher than the value of Noiler but showed no significant ( $p > 0.05$ ) difference when compared to the values of IB and LC. There were however no significant ( $p > 0.05$ ) variations in the mean low density lipoprotein cholesterol (LDL-C), total bilirubin, direct bilirubin, indirect bilirubin and creatinine concentrations among all the breeds investigated. In conclusion, the results obtained in this study will be helpful in creating a baseline data on biochemical profiles of indigenous and exotic chicken breeds in Nigeria which in turn can be used for assessing the health status of these birds, improving desirable breeds/traits and designing appropriate breeding strategies for indigenous poultry birds in the country..

**Keywords:** Bilirubin, domestic fowls, genetic variations, lipids, renal function markers

### INTRODUCTION

The assessment of the nutritional and health status in livestock can be made by determining certain blood metabolite concentrations (Ndlova *et al.*, 2007). The physiological status of an animal, breed, nutrition, season and age may affect the concentration of blood biochemical parameters. Blood plays an important role in the transportation of nutrients, metabolic waste products and gases around the body (Zhou *et al.*, 1999) and represents a means of assessing clinical and nutritional health status of animals (Olorode & Longe, 2000). The haemato-biochemical profiles are most commonly used in nutritional studies for chickens (Adeyemiet *et al.*, 2000) and other birds like pigeon (Pavlakiet *et al.*, 2005), guinea fowl (Onyeausi, 2007), bronze turkey (Schmidt *et al.*, 2009) and Japanese quail (Arora, 2010). It has been shown that data from blood profiles could be exploited in the improvement of chicken stocks

(Ladokunet *et al.*, 2008). In addition, blood parameters help in the diagnoses of specific poultry pathologies and might serve as basic knowledge for studies in immunology and comparative avian pathology (Bonadiman *et al.*, 2009).

Studies on Thai native chickens (Koronowicz *et al.*, 2016), naked-neck indigenous chickens of Kashmir (Pampori & Iqbal, 2007), Cobb broilers (Barreiro *et al.*, 2009; Daneshyar *et al.*, 2009) and laying hens (El-Gendy *et al.*, 2011; Yanagita *et al.*, 2011) showed that haemato-biochemical profiles of chickens are correlated with a number of factors such as gender, nutrition, rearing temperature, stocking density and stress conditions.

Total cholesterol is a precursor of steroid hormones and a simultaneously building component of cell membranes (Pavlik *et al.*, 2007; Zhang *et al.*, 2019).

Several factors (sex, breed, physiologic status, nutrition, age, genetics, stress, disease, management system, location and

season) are known to affect haemato-biochemical values in domestic animals (Addass *et al.*, 2010). Various reports have underlined the emerging observation that it may be difficult to formulate a universal biochemical profile for indigenous chickens due to interplay of the aforementioned factors (Daramola *et al.*, 2005; Addass *et al.*, 2010). Much work has not been done on the influence of breed on the serum biochemical values of laying chickens in the tropical zone of Abia State. Therefore, this study was designed to evaluate the effect of breed on the bilirubin levels, renal function markers and lipid profile of laying domestic chickens kept in the tropical eco-zone of Nigeria.

## MATERIALS AND METHODS

### EXPERIMENTAL ANIMALS

The study was carried out in the Poultry Unit of the Department of Animal Health and Production, College of Veterinary Medicine Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike. Sixteen (16) 18-week old apparently healthy Black Australorp (BA), Noiler, Isa brown (IB) and local chicken (LC) of mean weight  $1.99 \pm 0.12$  kg ( $1.85$ - $2.30$ kg) sourced from a poultry farm in the Southern Nigeria were used for this study. The birds (chickens) were acclimatized for three weeks before the commencement of this study. Appropriate routine prophylactic medications were given as and when due or when necessary to ensure the optimal health of the experimental chickens. The birds were fed twice daily with commercial layers mash throughout the period of the study. Clean fresh cool water was provided *ad libitum* for the chickens throughout the period of the study. Ethical approval for this study was obtained from the Ethical Committee of College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria (MOUAU/CVM/REC/202216).

### EXPERIMENTAL DESIGN

Sixteen (16) adult chickens were randomly assigned to four treatment groups (A to D) according to their genotypes of four (4) chickens per group in a completely randomized design (CRD). Group A: Black Australorp (BA), Group B: Isa Brown (IB), Group C: Noiler (NOI) and Group D: local chicken (LC). The birds were identified with tag letters thus; A<sub>1-4</sub>, B<sub>1-4</sub>, C<sub>1-4</sub> and D<sub>1-4</sub> representing groups A-D and they

were kept individually in separate cages and were maintained in these separate cages throughout the period of the study. Five milliliter (5ml) of blood samples were collected from the wing veins of the experimental birds (BA, IB, NOI and LC) and dispensed into plain sample bottles and allowed to clot in a slanting position and centrifuged at 2,500 RPM for 5 minutes (Hrubec *et al.*, 2004). The resulting sera were aspirated, stored at  $-20^{\circ}\text{C}$  and used to determine serum blood urea nitrogen (BUN) (Burtis & Ashwood, 1999), creatinine (Newman & Price, 1999), total bilirubin (Cheesbrough, 1991), direct bilirubin (Tietz, 1976), total cholesterol (TC) (Artiss & Zak, 1997), triacylglycerols (TAG) (Rifai *et al.*, 1999) and high density lipoprotein cholesterol (HDL-C) concentrations (Rifai & Warnick, 1994). Indirect bilirubin concentration was obtained by subtracting direct bilirubin from total bilirubin. Serum low density lipoprotein cholesterol (LDL-C) was also calculated using Friedewald's equation (Friedewald *et al.*, 1972).

$$\text{LDL-C} = [\text{TC} - \{\text{HDL-C} + (\text{TAG}/5)\}]$$

Serum very low density lipoprotein cholesterol (VLDL-C) concentration was calculated by multiplying TAG by 0.2 (Wilson *et al.*, 1981; Kenneth, 2001).

$$\text{VLDL} = 0.2 \times \text{TAG (where TAG is triacylglycerols)}.$$

### DATA ANALYSIS

The data collected for each of the serum renal function markers, bilirubin and lipid parameters from the different genotypes were presented as mean  $\pm$  SEM and subjected to One Way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS, 2012). Variations in means were separated using Duncan's New Multiple Range Test (Steel & Torrie, 2006; SAS, 2010). Probability values  $\leq 0.05$  were considered significant.

### RESULTS

The mean total cholesterol (TC), triacylglycerol (TAG) and very low density lipoprotein cholesterol (VLDL-C) concentrations of local chicken (LC) were significantly ( $P < 0.05$ ) higher than the Isa brown (IB) value. Similarly, the

**Table I: The mean serum bilirubin levels and kidney function markers of laying chickens as influenced by genotypes (breeds). Values are expressed as means  $\pm$  SEM in the table**

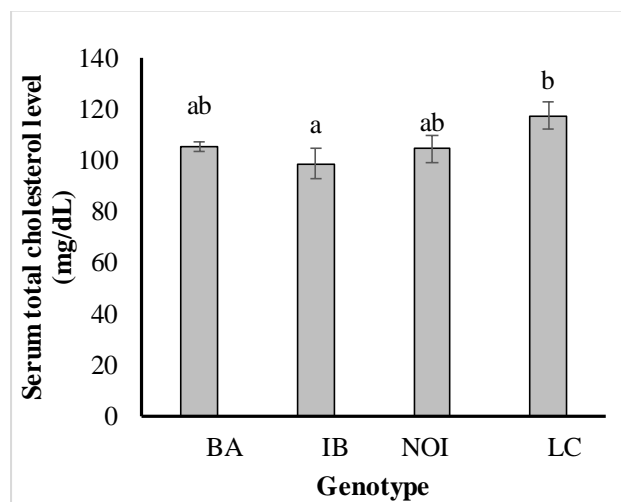
Parameters (mg/dl)	Treatments (Genotypes)			
	BA	IB	NOI	LC
Total Bilirubin	$0.33 \pm 0.02$	$0.32 \pm 0.01$	$0.33 \pm 0.01$	$0.38 \pm 0.04$
Direct Bilirubin	$0.14 \pm 0.02$	$0.14 \pm 0.03$	$0.16 \pm 0.04$	$0.12 \pm 0.02$
Indirect Bilirubin	$0.19 \pm 0.02$	$0.18 \pm 0.04$	$0.17 \pm 0.03$	$0.26 \pm 0.04$
Urea (BUN)	$12.60 \pm 0.51^{ab}$	$11.11 \pm 0.35^{ab}$	$9.77 \pm 0.94^a$	$13.18 \pm 1.39^b$
Creatinine (SCr)	$0.35 \pm 0.08$	$0.31 \pm 0.03$	$0.32 \pm 0.03$	$0.38 \pm 0.06$

<sup>ab</sup>Mean values in the same row with no similar superscripts are significantly different ( $P < 0.05$ )

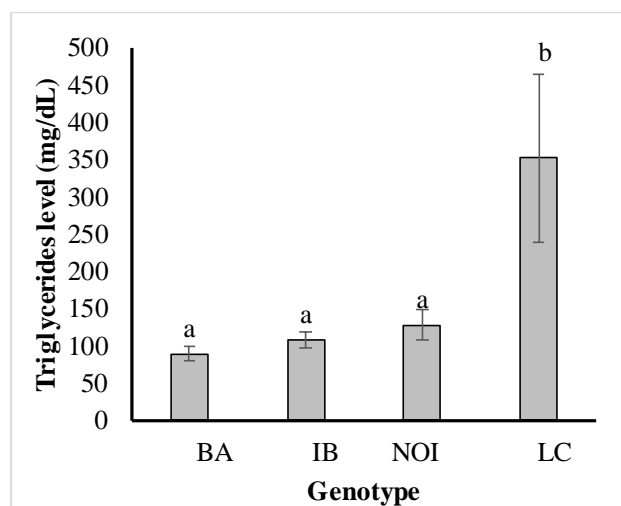
BA = Black Australorp; IB = Isa Brown; NOI = Noiler; LC = Local Chicken

mean blood urea nitrogen (BUN) concentrations of LC was significantly ( $P < 0.05$ ) higher than the Noiler (NOI) value.

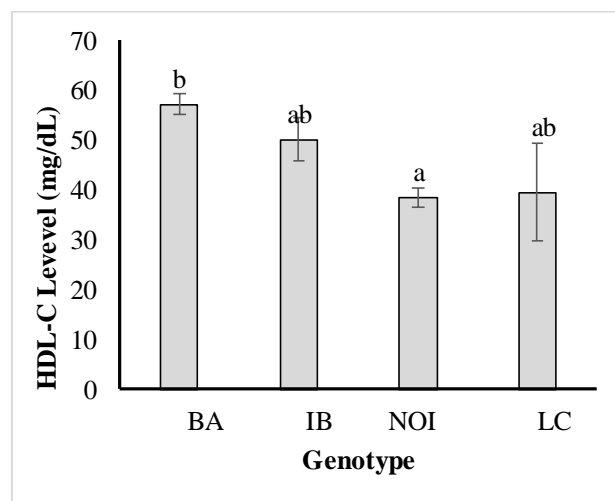
The mean high density lipoprotein cholesterol (HDL-C) of Black Australorp (BA) was significantly ( $P < 0.05$ ) higher than the value of Noiler but showed no significant ( $p > 0.05$ ) difference when compared to the values of IB and LC. There was however no significant ( $p > 0.05$ ) variations in the mean low density lipoprotein cholesterol (LDL-C), total bilirubin, direct bilirubin, indirect bilirubin and creatinine concentrations among all the breeds investigated.



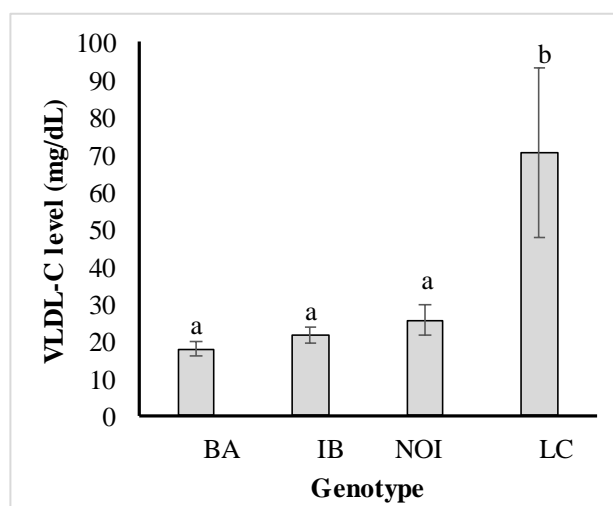
**Figure I: The mean serum total cholesterol concentration of laying chickens as influenced by genotypes (breeds).** Values are expressed as means  $\pm$  SEM. Values with different superscripts are statistically ( $p < 0.05$ ) different. BA = Black Australorp; IB = Isa Brown; NOI = Noiler; LC = Local Chicken



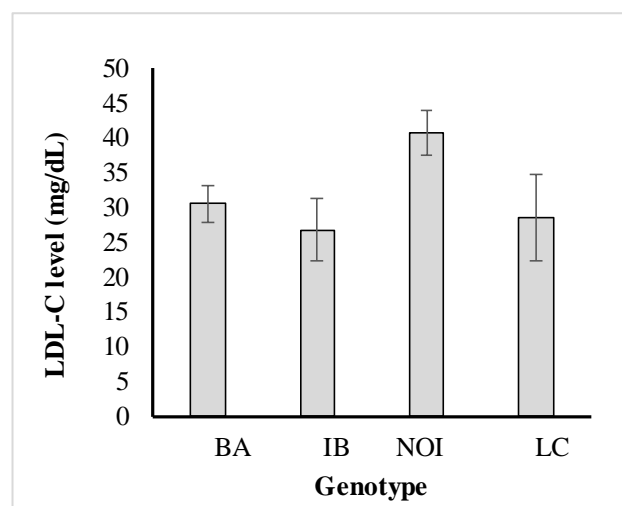
**Figure II: The mean serum triglycerides (TAG) concentration of laying chickens as influenced by genotypes (breeds).** Values are expressed as means  $\pm$  SEM. Values with different superscripts are statistically ( $p < 0.05$ ) different



**Figure III: The mean serum high density lipoprotein cholesterol (HDL-C) concentration of laying chickens as influenced by genotypes (breeds).** Values are expressed as means  $\pm$  SEM. Values with different superscripts are statistically ( $p < 0.05$ ) different



**Figure IV: The mean serum very low density lipoprotein cholesterol (VLDL-C) concentration of laying chickens as influenced by genotypes (breeds).** Values are expressed as means  $\pm$  SEM. Values with different superscripts are statistically ( $p < 0.05$ ) different.



**Figure V: The mean serum low density lipoprotein cholesterol (LDL-C) concentration of laying chickens as influenced by genotypes (breeds).** Values are expressed as means  $\pm$  SEM. Values with different superscripts are statistically ( $p < 0.05$ ) different.

## DISCUSSION

The results of the effect of genetic variations on the kidney function markers and bilirubin profiles of laying chickens are presented in Table I while the results of the effect of genetic variations on the lipid profile of laying chickens are shown in Figures I-V. Creatinine and urea are often used as indicators of renal function in mammals with elevations in both parameters above the reference ranges being seen in the latter stages of renal failure (Jenkins, 2008; Kaneko *et al.*, 2008). The urea values obtained in this present study were higher than the range of 0.48 to 0.63 mg/dl reported by Okpe & Abdulfatai (2022) for broiler chickens. Also, the blood urea nitrogen (BUN) values obtained in this present study were higher than the range of  $5.56 \pm 0.29$  to  $6.63 \pm 0.31$  mg/dl reported by Nweze (2021) in Duroc sows. However, the urea values obtained in this study were within the range of 10.20 to 29.74 mg/dl reported by Egu (2017) in Harco cocks except for the urea value of Noiler chickens which falls below this range. This disparity in urea values in previous works compared to this present study may be attributed to differences in breed and nutritional status of the birds/animals used for the study, management, climatic factors, among others. It has been observed that serum urea concentration depends on both the quantity and quality of protein supplied in the diets (Iheukwumere & Herbert, 2002).

The serum creatinine values obtained in this study were below the ranges of 1-2 mg/dl reported for birds by Banerjee (2007), 18.00-18.50 mg/100ml reported by Iheukwumere *et al.* (2002) in broiler chickens and  $2.46 \pm 0.09$  to  $2.86 \pm 0.28$  mg/dl reported in Duroc sows (Nweze, 2021). Creatinine measurement is used exclusively in the assessment of kidney

function. The rate of production of creatinine is constant and elevations of plasma creatinine are indicative of under excretion suggesting kidney impairment. Stockham & Scott (2007) reported also that creatinine along with blood urea nitrogen concentration is an excellent indicator of protein metabolism and kidney function.

Cholesterol and triacylglycerol in this present study showed significant breed variations. The cholesterol values obtained in this study were within the normal physiological range of 52-148 mg/dl reported by Banerjee (2007) for birds. This implies that the chickens used in this study may not face the risk of myocardial infarction usually associated with high blood cholesterol concentrations or emaciation due to low serum cholesterol concentrations (Frandsen, 2002). A decrease in the plasma cholesterol concentration has been reported to result in reduction in the plasma concentrations of insulin-like growth factor and progesterone and consequently delayed or inhibited ovulation (Maciele *et al.*, 2010).

## CONCLUSION

Genotypic variations cause statistically significant alterations in the blood urea nitrogen and lipid profile of domestic chickens used in this study. However, the magnitude of the observed decline in some serum metabolites is sufficiently great to suggest that breed of the chickens should be taken into consideration in setting routine upper reference limits for these measurements.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## REFERENCES

- Addass, P.A., Midau, A. & Babale, D.M. (2010). Haemato-biochemical findings of indigenous goats in Mubi, Adamawa State, Nigeria. *Journal of Agriculture & Social Sciences*, 6(1), 14-16.
- Adeyemi, O.A., Fasina, O.E. & Balogun, M.O. (2000). Utilization of full fat Jatropha seeds in broiler diet: Effect on haematological parameters and blood chemistry. *Proceeding of the 25th Conference of the Nigerian Society for Animal Production held at Michael Okpara University of Agriculture, Umudike, Nigeria, March 19-23*, pp.108 - 109.
- Arora, K.L. (2010). Differences in haemoglobin and packed cell volume in blood collected from different sites in Japanese quail (*Coturnix japonica*). *International Journal of Poultry Science*, 9(9), 828-830.
- Artiss, J.D. & Zak, B. (1997). Measurement of cholesterol concentration. In: Rifai N, Warnick GR, Dominiczak MH, eds. *Handbook of lipoprotein testing*. Washington, AACC Press, p 99-114.
- Atansuyi, A.J. & Chineke, C.A. (2011). Relationship between packed cell volume and other haematological parameters of rabbits fed graded levels of fibre sources. *16th Annual Conference Proceeding of the Animal Science Association of Nigeria at the Kogi State Univ. Ayingba, Kogi State, Nig.* Page 153 - 155.

- Banerjee, G.C. (2007). *A Textbook of Animal Husbandry* (8th edition). Oxford and IBH Publishing co PVT Ltd New Delhi India.
- Barreiro, F.R., Sagula, A.L., Junqueira, O.M., Pereira, G.T. & Baraldi-Artioni, S.M. (2009). Densitometric and biochemical values of broiler tibias at different ages. *Poultry Science*, 88(12), 2644-2648.
- Bonadiman, S.F., Stratievsky, G.C., Machado, J.A., Albernaz, A.P., Rabelo, G.R. & Damatta, R.A. (2009). Leukocyte ultrastructure, haematological and serum biochemical profiles of ostriches (*Struthio camelus*). *Poultry Science*, 88(11), 2298-2306.
- Burtis, C.A. & Ashwood, E.R. (1999). editors. *Tietz Textbook of clinical Chemistry*. 3rd ed. Philadelphia: W.B Saunders company; p. 1838.
- Cheesbrough, M. (1991). *Medical Laboratory Manual for Tropical Countries*. ELBS. Ed. pp. 465-545.
- Daneshyar, M., Kermanshahi, H. & Golian, A. (2009). Changes of biochemical parameters and enzyme activities in broiler chickens with cold-induced ascites. *Poultry Science*, 88(1), 106-110.
- Daramola, J.O., Adeloje, A.A., Fatoba, T.A. & Soladoye, A.O. (2005). Haematological and biochemical parameters of West African Dwarf goats. *Livestock Research for Rural Development*. <http://www.lrrd.org/lrrd17/8/dara17095.htm>, retrieved 16-04-2015.
- Egu, U.N. (2017). Haematological and Serum Biochemical Parameters of Mature Harco cocks Treated with Human Menopausal Gonadotrophin (Diclair®) for Spermatogenesis. *International Journal of Environment, Agriculture & Biotechnology*, 2(1), 429-436.
- EL-Gendy, E.A., EL-Komy, E.M., EL-Far, A.A., EL-Gamry, K.A. & EL-Mallah, G.M. (2011). Developmental stability in chickens local to warm climatic region. 2. Variation in blood metabolites due to genetic selection and crossing. *International Journal of Poultry Science*, 10(5), 358-364.
- Frandsen, R. D. (2002). *Anatomy and Physiology of farm animals 3rd ed. Published by Bialiere Tindal, London*, Pp. 32-54.
- Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clinical Chemistry*, 18, 499-502.
- Hrubec, T.C., Whichard, J.M., Larsen, C.T. & Pierson, F.W. (2004). Plasma versus serum: Specific differences in biochemical analytic values. *Journal of Avian Medicine and Surgery*, 16(2): 101-105.
- Iheukwumere, F.C. & Herbert, U. (2002). Physiological responses of broiler chickens to quantitative water restriction. Haematology and Serum Biochemistry. *International Journal of Poultry Science*, 2(2), 117-119.
- Iheukwumere, F.C., Herbert, U. & Ewulu, C. (2002). Effect of quantitative feed restriction on broiler chickens. *Journal of Sustainable & Tropical Agriculture Research*, 4, 56-60.
- Jenkins, J.R. (2008). Rodent diagnostic testing. *Journal of Exotic Pet Medicine*, 17, 16-25.
- Kaneko, J.J., Harvey, J.W. & Bruss, M.L. (2008). Appendices In: Kaneko JJ, Harvey JW, Bruss ML, editors. *Clinical biochemistry of domestic animals*. 6th edition. San Diego California: Academic Press; pp. 889-895.
- Kenneth, D.M. (2001). *Clinical laboratory medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams and Wilkins.
- Koronowicz, A.A., Banks, P., Szymczyk, B., Leszczyńska, T., Master, A., Piasna, E., Szczepański, W., Domagała, D., Kopeć, A., Piątkowska, E. & Laidler, P. (2016). Dietary conjugated linoleic acid affects blood parameters, liver morphology and expression of selected hepatic genes in laying hens. *British Poultry Science*, 57, 663-673.
- Ladokun, A.O., Yakubu, A., Otite, J.R., Omeje, J.N., Sokunbi, O.A. & Onyeji, E. (2008). Haematological and serum biochemical indices of naked neck and normally feathered Nigerian indigenous chickens in a sub humid tropical environment. *International Journal of Poultry Science*, 7(1), 55-58.
- Maciel, M., Marais, S.M., Bevilacqua, C.W., Silva, R.A., Baros, R.S., Sousa, R.N., Sousa, L.C., Brito, E.S. & Sousa-Neto, M.A. (2010). Chemical Composition of *Eucalyptus spp*, essential oils and their insecticidal effects on *Lutzomyia longipalpis*. *Veterinary Parasitology*, 167(1), 1-7.
- Ndlova, T., Chimonyo, M., Okoh, A.J., Muchenje, V., Dzama, K. & Racts, J.G. (2007). Assessing the nutritional status of beef cattle; current practices and future prospects. *African Journal of Biotechnology*, 6(24), 2717-2734.
- Newman, D.J. & Price, C.P. (1999). Renal function and nitrogen metabolites. In: Burtis CA, Ashwood ER, editors. *Tietz Textbook of Clinical Chemistry*. 3rd ed. Philadelphia: W.B Saunders Company; p. 1204-1270.
- Nweze, E.C. (2021). Endocrine profiles and kidney function markers of apparently healthy pregnant and lactating Duroc sows. *Journal of Sustainable Veterinary & Allied Sciences*, 1(2), 162-166.
- Okpe, A.A. & Abdulfatai, T. (2022). Serum biochemistry of broiler chickens fed different levels of pawpaw leaf meal (PLM) in the diet. *GSC Biological & Pharmaceutical Sciences*, 19(02), 233-238.
- Olorode, B.R. & Longe, O. G. (2000). Effect of replacing palm kernel cake with shear butter cake on quality characteristics, haematology and serum chemistry of laying hens. *Nigerian Journal of Animal Production*, 27, 19-23.
- Onyeausi, B.I. (2007). Calcium and phosphorus levels in Nigerian guinea fowls. *International Journal of Poultry Science*. 6(8), 610 - 611.
- Pampori, Z.A. & Iqbal, S. (2007). Haematology, serum chemistry and electrocardiographic evaluation in native chicken of Kashmir. *International Journal of Poultry Science*, 6(8), 578-582.

- Pavlak, M., Vlahovic, K., Jarcic, J., Dorc, A. & Zupancic, Z. (2005). Age, sexual and seasonal differences of haematological values and antibody status to *Chlamydophila sp.* in feral and racing pigeons (*Columba livia* forma domestica) from an urban environment (Zagreb, Croatia). *European Journal of Wildlife Research*, 51(4), 271-276.
- Pavlik, A., Pokludová, D., Zapletal, P. & Jelínek, P. (2007). Effects of housing systems on biochemical indicators of blood plasma in laying hens. *Acta Vet. Brno.*, 76, 339-347.
- Raney, T., Gerosa, S., Skoet, K., Skoet, J., Steinfeld, H., McLeod, A., Opio, C. & Cluff, M. (2009). *The state of food and agriculture 2009, Livestock in Balance*, FAO
- Rifai, N. & Warnick, G.R. (1994). Ed. *Laboratory Measurement of Lipids, Lipoproteins and Apolipoproteins* AACC Press. Washington, DC, USA.
- Rifai, N., Bachorik, P.S. & Albers, J.J. (1999). Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. *Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company*, p. 809-61.
- Schmidt, E.M.S., Paulillo, A.C., Martins, G.R.V., Lapera, I.M., Testi, A.J.P., Junior, L.N., Denadai, J. & Fagliari, J.J. (2009). Haematology of the bronze turkey (*Meleagris gallopavo*): Variations with age and gender. *International Journal of Poultry Science*, 8(8), 752-754.
- Statistical analysis system (SAS). (2010). *SAS user's guide*. Cary, NY: SAS institute.
- Statistical Package for Social Sciences (SPSS). (2012). *International Businesses machines of Statistical Package for Social Sciences Statistics for Windows*, Version 21.0. IBM Corp., Armonk, NY.
- Steel, R.D.G. & Torrie, J.H. (2006). *Principles and Procedures of Statistics. 3rd. Edn. McGraw-Hill Book Co. Inc., New York*.
- Stockham, S.L. & Scott, M.A. (2002). *Fundamentals of Veterinary Clinical Pathology*. Iowa State University Press, Ames. I. A. USA.
- Tietz, N.W. (1976). *Fundamentals of Clinical Chemistry. W.B. Saunders Co., Philadelphia*, p.1040.
- Wilson, P.W., Abbott, R.D., Garrison, R.S. & William, P.C. (1981). Estimation of very low density lipoprotein cholesterol from data on triglyceride concentration in plasma. *Clinical Chemistry*, 27, 2008 – 2010.
- Yanagita, K., Shiraishi, J., Kawakami, S. & Bungo, T. (2011). Time course changes in the blood parameters and the expression of diencephalic CRH and AVT mRNA due to acute isolation stress in chicks. *Journal of Poultry Science*, 48(2), 125-129.
- Zhang, J., Li, Q., Wu, Y., Wang, D., Xu, L., Zhang, Y., Wang, S., Wang, T., Liu, F., Zaky, M.Y., Hou, S., Liu, S., Zou, K., Lei, H., Zou, L., Zhang, Y. & Liu, H. (2019). Cholesterol content in cell membrane maintains surface levels of ErbB2 and confers a therapeutic vulnerability in ErbB2-positive breast cancer. *J. Cell Commun. Signal.*, 17, 15.
- Zhou, W.T., Fujita, M. & Tammamto, S. (1999). Thermoregulatory responses and blood viscosity in dehydrated heat-exposed broilers (*Gallus domesticus*). *Journal of Thermal Biology*, 24(3), 185-192.