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Variation in the ability of Iraqi local chickens to resist inflammation through an analytical study of the physiological properties of blood

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Abstract

The scarcity of information reduces the genetic ability to select chickens that are resistant to infection, and because the Iraqi local chicken is one of the important economic components of rural families. So, it is necessary to work to increase its resistance to all forms of infection. The current study aims to make a comparison between local chicken breeds in the severity of their resistance to infections by studying physiological blood traits. All vital blood tests were performed for male and female repeats, white, black and striped breeds .The results of the study confirmed the existence of statistically significant differences ($P \leq 0.05$) between the studied breeds in some vital and enzymatic blood traits, which indicates the confirmation of the existence of a discrepancy between the breeds even though they were all subject to the same environmental and nutritional treatments.

Keywords: **Local chickens, Blood traits, SOD, H_2O_2**.

Introduction

Chicken is a domestic fowl and is one of the most common and widespread domestic pets (Monckton et al., 2020) The increased consumption of protein derived from poultry requires greater poultry production (Fouad et al., 2020). The local chicken is a biodiversity that plays an important role in maintaining the sustainability of the ecosystem (Fatmona et al., 2020). The local chicken is important in rural areas because it is considered a meat and egg product without any nutritional additives and because of its high resistance to diseases and good adaptation to the environment (Msoffe et al., 2002; Bahmanimehr, 2012). Infection poses a major threat to chicken production and a major problem for rural households, as this infection limits the contribution of chicken production to improving the livelihoods of poor rural communities (Mpenda et al., 2019). Selection of chickens that are resistant to infection and viral is a promising approach to control losses caused by infections (Bacon et al., 2000). Methods for detecting infections in chickens were limited to veterinary practices only, but now modern techniques and scientific applications lead to the formation of a clear and early picture on the physiological responses of the body and of high value in understanding and studying infections (Stevenson et al 2005) .

The principles of selection that were adopted in chickens focused on the characteristics of early growth rate and efficiency of food conversion, while a high degree of attention was not paid to the traits of resistance to diseases and infections, which ultimately led to the lack of clear criteria for physiological characteristics and consequently the contradiction in the balance of the different functional components of the immune system (Marx et al. 1985). The cause of the death of many chicken breeds is due to metabolic disorders, some of which have been found to have a response more susceptible to disease and infections (Shapiro et al. 1998). This is due to impaired adaptive immune responses (Qureshi et al., 1994). On the other hand, selection based on improving immunocompetence was found to result in lower growth rates (Siegel et al. 1987). There are two functional arms of the immune system, the first is natural innate immunity and the second is acquired immunity, as it was believed that innate immunity was the first line of defense and worked to reduce inflammation until the emergence of acquired immunity (Goldsby et al., 2000). However, recent studies confirm that the innate immune response serves to provide instructions for the acquired immune response (Fearon et al., 1996; Parish et al., 1997). Recent studies are discovering several alternative antibiotic strategies in order to enhance the innate immunity of poultry and reduce the use of antibiotics (Lillehoj et al., 2012). Changing environmental conditions have the ability to change an animal's susceptibility to, and resistance to, infectious and inflammatory agents. It is important to know how these stresses affect the animal's immune system through physiological blood parameters (Hangalapura et al., 2003). Blood tests can serve as a useful tool for identifying chickens susceptible to infection and at risk for coccidiosis, allowing for better preventive measures (Adenaike et al., 2018). In addition, hematological and biochemical parameters serve to provide value on the immune status of chickens (Ladokun et al., 2008). So, the aim of this study was to resist inflammation in Iraqi local chickens by analytical physiological of blood.

Materials and Methods

Blood samples were taken from three breed Iraqi local chicken for the 18 replicates from the pterygoid vein and placed in special transparent glass tubes that were prepared in advance. Divieded into Black male (Bm), Black female (Bf), White male (Wm), White females (Wf), Striped male (WBm), Striped female (WBf) .

Blood was drawn using medical syringes (5) ml, and the blood taken was divided into two parts, where the first part was placed in containers (plastic tubes). It contains EDTA, which prevents clotting, and the second part of the sample was placed in containers (plastic tubes) devoid of anticoagulant. After completion, the samples (the two types) were placed in a refrigerated plastic container containing an amount of ice for the following biochemical tests.

Red blood cell count calculations: Red cell counts were performed in the laboratory according to the method of after dilution only (Natt & Herrik, 1952), by applying the following equation :

The number of red blood cells in a cubic milliliter of blood= $N/5 \times 25 \times 200 \times 10$

N= Sum of cells calculated in 5 large squares (80 small squares).

5 =the number of large squares that have been counted inside

25 =the total number of big squares

200 =the number of times the blood is diluted

10 =The number obtained is multiplied by 10 to represent the number of red blood cells in 1 cubic milliliter of blood

White Blood Cell count: According to the Campbell method 1995 by applying the following equation :

WBC count in 1 mm^3 of blood = (Number of leukocytes in 9 large squares +10% of total leukocyte count) x 200

Aggregated Blood Cell Volume Measurements PCV%: The volume of compacted blood cells was measured as a percentage, according to the Campbell method 1995. By drawing a blood sample using a capillary tube open from both sides, where this tube is immersed in the blood sample, and after the blood in this capillary tube rises to two-thirds of its length (capillary characteristic), its end is closed with artificial mud. then but this tubes in a horizontal microcentrifuge at 12,000 rpm for 7-14 minutes. The reading is carried out using a special ruler prepared for this purpose.

Hemoglobin Measurements Hb: To calculate the concentration of hemoglobin in blood samples, Campbell 1995 equation was adopted, through a direct method, based on measurements of the PCV stacked blood cell volume.

Hemoglobin concentration (g/100ml) = (Volume PCV /3.3)

Protein Calculations: The total blood protein of the samples was calculated by applying the method Coles (1986). By applying the following equation:

Total protein concentration g/di = (sample uptake rate / standard solution uptake rate) x 60 (standard solution concentration).

Albumin Concentration Calculations:

Albumin concentration (g/dl) = (absorbance of the sample / absorbance of the standard solution) x 5 g

Glucose Measurements: A spectrophotometer was used to read the samples with a wavelength of 546 nm and the following equation was applied:

Glucose (mg/di) = (sample reading / control reading) x 100

Cholesterol Measurements: by adopting method Allain (1974) the readings were recorded using a spectrophotometer with a wavelength of 500 nm and by applying the following equation:

Cholesterol concentration (Mg/dl) = (Sample absorption rate / Standard solution absorption rate) x Standard solution concentration .

Measurement of ALP (Alkaline Phosphatase) in serum: The activity of ALP basal phosphatase was calculated in the serum of the research replicates according to the instructions prepared by Zarnab et al. 2019 .

G.O.T. (Glutamic Oxaloacetic Transaminase) enzyme activity measurement: The activity of the GOT enzyme was measured in the serum of all the research replicates by using the measurement solutions of the color method of the reagent Dinitrpheny1 hydrazine 2.4, which was prepared according to the method approved by Hussain 2019

Measurement of G.P.T. (Glutamat Pyruvic Transaminase) enzyme activity: The activity of the GPT enzyme was measured in the serum of all the research replicates by using the measurement solutions of the color method of the reagent Dinitrpheny1 hydrazine 2.4, which was prepared according to the method approved by Hussain (2019).

Measurement of the activity of the enzyme SOD (superoxide dismutase) in the blood serum: Made by adopting method Fridovich 1989.

Measuring the effectiveness of H_2O_2 (Hydrogen peroxide): To measure the hydrogen peroxide concentration in blood samples, the steps of the (Yavuz & Pelit, 2020) method were adopted, where a complex solution consisting of (FeIII, EDAT, NH3) was prepared to obtain a complex reagent in the blood. To complete this process, we used 0.209 g of solid compound (6H2O, Fecl3) which was then transferred to a glass beaker where it is dissolved in 10 ml of double distilled water. Then clean after 5.2 g by weight of the solid compound (EDTA, H2, Na2) to the components of this mixture and the mixing process was continued until the compound was completely dissolved, then after this process we added 10 ml of 25% blood (w/w) of NH3 solution to the mixture. In order to obtain an alkaline medium and at the end 0.204 ml of solid Na 2S2, O 3 is added to the mixture and diluted to 25 ml in a volumetric glass beaker. For the measurement method, all measurements of this process were made in a 3.5 ml quartz cell (tube) using a spectrophotometer. Then, the quartz cell was cleaned with 0.1 M HCl, then the cell was rinsed with very pure water and before analyses were performed. The cell was treated with acetone and then dried with 99.9% pure nitrogen to remove the water and acetone remaining within it. Then we added 2 ml of the sample in which concentrated H and 1.2 ml of concentrated NH 3 was added to the quartz cell, then we added 300 µl of the complex reagent to the cell, and then the solution was mixed well for 2 seconds. Where the purple-colored peroxo compound was directly formed, spectroscopic measurements were taken at 525 nm in a 5-s period, readable thermometers were taken against the blank solution and a standard curve was made. The method of sample analysis is to filter the blood samples through filter paper to get rid of the particles present in the blood samples before analysis. Samples were analyzed and the readings were presented on the standard curve to obtain the final hydrogen peroxide concentration.

Statistical analysis: Statistical analysis of the data in this study was carried out by following the steps of the SPSS statistical program and comparing the significant differences (ANOVA) between the mean values by adopting the (Duncan1955) multinomial test at the level of significance ($P \leq 0.05$), Where the effect of the coefficients for the studied traits was studied using the complete random design (CRD).

Results and Discussion

The results of the statistical analysis in (Table 1) showed that black females significantly outperformed at the level ($P \leq 0.05$) on all treatments of the experiment in the characteristic of total protein concentration in serum and there was no significant difference between them .

As for the cholesterol concentration in the blood, the results of the statistical analysis showed that the black Bm males were significantly outperformed at the level ($P \leq 0.05$) over all the treatments of the experiment, and there was no significant difference between them. While, the results of the concentration of uric acid showed that the black males were significantly superior at ($P \leq 0.05$) over the white females and the striped males, while there were no statistically significant differences between the rest of the treatments. As for the concentration of glucose in the blood, the results of the statistical analysis of this study showed that black females and striped females were significantly ($P \leq 0.05$) superior to black males, black striped males and white males. While there were no significant differences between white females and striated females ($p \leq 0.05$). Also, there were no significant differences at the level ($p \leq 0.05$) between black males, striped males, and white males.

The results of the statistical analysis of albumin concentration in the blood showed that black females were significantly superior at ($P \leq 0.05$) over all the treatments of the experiment. Also, significantly outperformed striped males and white males, and white females outperformed white males. There are no statistically significant differences between them.

Table (1): Show the traits of protein, cholesterol, uric acid, glucose, and albumin in Iraqi local chicken breeds (Means ± Standard Error)

Adjective / Breeds	Protein g/dl	Cholesterol Mg/dl	Uric	Glucose Mg/dl	Albumins g/dl
Bf	5.66 ± 0.03 a	216.00 ± 2.80 c	4.16 ± 0.03 ab	198.00 ± 0.01 a	3.66 ± 0.03 a
Wf	5.60 ± 0.00 ab	214.33 ± 0.33 cd	4.13 ± 0.03 b	196.00 ± 0.57 ab	5.33 ± 0.06 abc
WBf	5.53 ± 0.03 bc	213.00 ± 0.57 d	4.20 ± 0.00 ab	196.66 ± 0.88 a	3.60 ± 0.05 ab
Bm	5.53 ± 0.02 bc	223.33 ± 1.2 a	4.26 ± 0.03 a	194.33 ± 0.00 bc	3.50 ± 0.05 bcd
Wm	5.46 ± 0.03 cd	221.66 ± 0.88 ab	4.23 ± 0.03 ab	192.33 ± 0.66 c	3.36 ± 0.03 d
WBm	5.40 ± 0.00 d	219.66 ± 0.33 b	4.13 ± 0.03 b	193.00 ± 1.00 c	3.40 ± 0.00 cd

Different lowercase letters indicate significant differences at the level of (P≤ 0.05), Black female (Bf), White females(Wf), Striped female(WBf), Black male(Bm), White male(Wm), Striped male(WBm) .

The difference in serum total protein concentrations between study breeds can be due to differences in genetic structures, and these differences may be due to many factors that may be endogenous or exogenous, which can occur due to the physical roles of blood proteins .

The increase in total protein and albumin is a direct consequence of the increased need for amino acids required for the growth of the bird's body (Szabo et al., 2005). The difference in albumin concentrations may be due to the age of the birds (60 weeks) or to the different genotypes of the study lines, where albumin is an extracellular antioxidant. Where Halliwell et al (2015) pointed out the effect of albumin on antioxidants through its inhibition of free radicals, and there is a possibility that the protein concentration and high albumin content is a result of the bird’s performance and the need for amino acids for its growth (Szabo et al. 2005) . Whereas Halliwell et al (2015) showed that albumin inhibits free radicals through its association with hydraulic compounds and fatty acids. As for the concentration of glucose and cholesterol, significant differences were observed between the study breeds for this trait, due to the different genotypes between the study breeds or the high percentage of carbohydrate CHO as a result of feeding.

The results of the statistical analysis (Table 2) showed that there were statistically significant differences at the level (P≤ 0.05) of the study samples in the cellular characteristics of the blood. With regard to the proportion of PCV agglutinated blood cells, black males significantly outperformed all treatments of the experiment . As for the level of hemoglobin in the blood, the statistical analyzes showed that there were no statistically significant differences between black and striped males. While there were statistically significant differences at the level (P≤ 0.05) between black males and other breeds.

Regarding the number of red blood cells, black males were significantly superior to all study replicates, while black females outperformed white and striped males and white and striped females. While there are no significant differences between white and striped males, and there are no significant differences between white females and white males and striped females. Regarding white blood cell numbers, white and black males were significantly outperformed by white females, while there were no significant differences between replicates for the rest of the study.

Table (2): The percentages of the aggregated blood cells, hemoglobin, and the numbers of red and white blood cells in Iraqi local chicken breeds (means ± standard error)

Breeds \ Adjective	PCV %	HB	RBC	WBC
Bf	35.76 ± 0.03 b	8.13 ± 0.03 c	34.60 ± 0.00 b	24.53 ± 0.06 ab
Wf	35.50 ± 0.00 c	7.96 ± 0.03 d	34.36 ± 0.03 d	24.43 ± 0.06 b
WBf	35.33 ± 0.03 d	8.03 ± 0.03 cd	34.33 ± 0.03 d	24.53 ± 0.03 ab
Bm	35.96 ± 0.06 a	8.43 ± 0.03 a	34.80 ± 0.05 a	24.63 ± 0.08 a
Wm	35.70 ± 0.05 b	8.26 ± 0.03 b	34.50 ± 0.00 c	24.63 ± 0.03 a
WBm	35.53 ± 0.03 c	8.33 ± 0.03 ab	34.50 ± 0.00 c	24.56 ± 0.03 ab

Different lowercase letters indicate significant differences at the level of (P≤ 0.05), Black female (Bf), White females (Wf), Striped female (WBf), Black male (Bm), White male (Wm), Striped male (WBm).

The discrepancy in PCV volume values may be due to the difference in the number of red blood cells, where Akinloye et al (2014) indicated that the relationship between red blood cells, Hb, and PCV is a direct relationship because it increases with its increase and decreases with its decrease. The variance may be due to differences in genotypes or to the age of study replicates. Saleh et al (2009) indicated a direct relationship between red blood cell count and (Hb, PCV) dependence on hematological parameters and in particular WBC can be an alternative method for measuring corticosterone, because any change in their numbers leads to an increase in glucocorticoid hormones and thus to changes in the immune system of birds (Romero et al 2005). The variability in the numbers of red blood cells obtained confirms the existence of differences between the study breeds, and this is an important marker for determining the physiological state of the animal. As a study for Pertille et al (2020) indicated that the variance in the numbers of red blood cells is a useful biomarker for identifying animals exposed to stress chronic in the production stage.

The results of the statistical analysis in (Table 3) at the significance level (P≤ 0.05) for the GOT enzyme showed that there were statistically significant differences between the treatments, where the black and striated females were significantly superior to the rest of the study treatments. While striped males and white females outperformed white males. There were no significant differences between striped males, white females, and black males, and there were no significant differences between black and white males, and there were no significant differences between black and striped females. Regarding the GPT enzyme results of the statistical analysis showed significant differences between the striated females and the white males. As for the rest of the study parameters, there were no statistically significant differences between them. Also, the results of AIP enzyme concentration, the statistical analysis showed significant differences between black and striped females. There are also statistically significant differences between black and striped females and white and striped males. As for the rest of the experimental treatments, there were no statistically significant differences between them.

Table(3): Activity of GOT, GPT, ALP enzymes in Iraqi local chicken breeds (Means ± Standard Error)

Breed \ Adjective	GOT	GPT	ALP
Bf	113.00 ± 0.57 a	13.33 ± 0.66 ab	37.33 ± 0.33 a
Wf	110.00 ± 0.33 b	12.66 ± 0.33 ab	35.00 ± 0.57 bc
BWf	113.33 ± 0.33 a	13.66 ± 0.66 a	36.00 ± 0.57 b
Bm	110.00 ± 0.57 bc	13.00 ± 0.57 ab	35.00 ± 0.57 bc
Wm	108.66 ± 0.33 c	11.66 ± 0.33 b	34.00 ± 0.00 c
WBm	111.00 ± 0.57 b	12.66 ± 0.33 ab	34.00 ± 0.00 c

Different lowercase letters indicate significant differences at the level of ($P \leq 0.05$), Black female (Bf), White females (Wf), Striped female (WBf), Black male (Bm), White male (Wm), Striped male (WBm).

Liver enzymes are an important biomarker to know the pathological changes that occur in hepatocytes (Gutierrez & Navarro, 2010). An increase in liver biomarkers indicates hyperactivity and evidence of apoptosis in the liver (Kourouma et al. 2015). where a decrease or increase in liver enzymes indicates a disease or abnormality in liver function, or a deficiency of the protein needed to produce these enzymes (Obeten et al. 2013). The obtained liver enzyme activities indicate a significant superiority in the liver activities of the black and striped female breeds, and this leads us to the higher incidence of apoptosis in these breeds than others.

The results of the statistical analysis in (Table 4) of Hydrogen peroxide H_2O_2 at a significant level ($P \leq 0.05$) indicated that white males significantly outperformed all treatments of female breeds, as well as the superiority of black males, striped, and white and black females significantly over striped females, while there were no significant differences between the rest of the treatments. While the SOD enzyme, statistical analysis showed that black females significantly outperformed white and striped males. The striated and white females outperformed the striated males in performance, while there were no statistically significant differences between the rest of the study parameters.

Reactive oxygen species (ROS) are critical determinants of cellular signaling and their levels must be carefully balanced in the body to ensure proper cellular function, survival, and the continuity of life. Therefore, SOD has a major and effective role in mitigating the adverse effects of ROS. SOD is the only extracellular enzyme with unique properties and functions in cellular signal transduction (Griess et al., 2017). The discrepancy in the activity of superoxide dismutase between the blood of study replicates may be indicative of an increase or decrease in the production of ROS and consequently a weakness in finding oxidative balance, as altered levels of it provide clear evidence of an antioxidant state in the body and thus are an indicator of the occurrence of oxidative stress (Georgieva et al., 2006).

The presence of statistical differences between the study breeds for the enzyme SOD and hydrogen peroxide H_2O_2 is an indicator of the presence of metabolic variations in chicken liver and thus can evaluate the relationship between the content of the diet and the metabolic changes that take place in the liver (XU et al 2014). High H_2O_2 concentrations lead to damage to pancreatic cells and thus affect insulin secretion and alter glucose metabolism (Naziragiu et al., 2012; Matsui et al., 2010). The SOD enzyme catalyzes the decomposition of H_2O_2, and the ratio of SOD is of great importance because it can indicate the ability of tissues to deal with oxidative stress (Bondy 1992). Therefore, study replicates that have good statistical significance for this trait, are a positive predictor.

Table (4): Hydrogen peroxide and superoxide dismutase enzyme activity in Iraqi local chicken breeds (Mean ± Standard Error)

Adjective / Breeds	H_2O_2	SOD
Bf	7.03 ± 0.03 b	8.60 ± 0.08 a
Wf	7.06 ± 0.08 b	8.56 ± 0.06 ab
WBf	6.76 ± 0.08 c	8.53 ± 0.06 ab
Bm	7.20 ± 0.11 ab	8.43 ± 0.08 abc
Wm	7.40 ± 0.05 a	8.40 ± 0.05 bc
WBm	7.10 ± 0.05 a	8.33 ± 0.03 c

Different lowercase letters indicate significant differences at the level of ($P \leq 0.05$), Black female (Bf), White females (Wf), Striped female (WBf), Black male (Bm), White male (Wm), Striped male (WBm).

Conclusions

There is a discrepancy in the physiological blood measurements between the studied strains that lived under the same environmental conditions, nutritional treatments and the same healthy age level. This discrepancy is due to the genetic specialization of each species.

Black line males outperformed in all vital blood traits.

The black female line and the striped female outperformed in all liver enzyme assays

References

Adenaike, A. S., Peters, S. O., Adeleke, M. A., Fafiolu, A. O., Takeet, M. I., & Ikeobi, C. O. N. (2018). Use of discriminant analysis for the evaluation of coccidiosis resistance parameters in chickens raised in hot humid tropical environment. *Tropical animal health and production*, *50*(5), 1161-1166.

Akinloye, O. A., Balogun, E. A., Omotainse, S. O., & Adeleye, O. O. (2014). Some untoward effects associated with the use of the biopreparation from Picralima nitida seeds extract as antidiabetic agent. *Biotechnologia Acta*, *7*(2), 92-105.

Allain, C. C.; Poon, L. S.; Chon, C. S. G.; Richmond, W. and Fu,P.C. 1974. Enzymatic determination of total serum cholesterol . Clin. Chem., 20: 470 – 475.

Bacon, L. D., Hunt, H. D., & Cheng, H. H. (2000). A review of the development of chicken lines to resolve genes determining resistance to diseases. *Poultry science*, *79*(8), 1082-1093.

Bahmanimehr, A. (2012). Selection for economic traits in chickens breeding program according to genetic parameters and correlation between traits. World Applied Sciences Journal, 20(10), 1332-1335.

Bondy SC (1992) Ethanol toxicity and oxidative stress. Toxicol Lett 63:231–241

Campbell, T. W. (1995). *Avian hematology and cytology* (No. Ed. 2). Iowa State University Press.

Coles, E. A. 1986. Veterinary clinical pathology. 4th edit. Sanders W. B. Co. Philadelphia. London – pp:124 - 127.

Duncan, D. B. (1955). Multiple range and multiple F tests. *biometrics*, *11*(1), 1-42.

Fatmona, S., & Nursjafani, N. (2020). Keanekaragaman Fenotipe Ayam Lokal (Gallus gallus domesticus) di KotaTernate. Cannarium, 18(1), 30-43.

Fearon, D. T., & Locksley, R. M. (1996). The instructive role of innate immunity in the acquired immune response. *Science*, *272*(5258), 50-54.

Fouad, A. M., El-Senousey, H. K., Ruan, D., Xia, W., Chen, W., Wang, S., & Zheng, C. (2020). Nutritional modulation of fertility in male poultry. Poultry Science, 99(11), 5637-5646.

Fridovich, I. (1989). Superoxide dismutases: an adaptation to a paramagnetic gas. Journal of Biological Chemistry, 264(14), 7761-7764.

Georgieva, N. V., Koinarski, V., & Gadjeva, V. (2006). Antioxidant status during the course of Eimeria tenella infection in broiler chickens. *The Veterinary Journal*, *172*(3), 488-492.

Goldspy, R. A., Kindt, T. J., & Osborne, B. A. (2000). In Kuby immunology 4th ed.

Griess, B., Tom, E., Domann, F., & Teoh-Fitzgerald, M. (2017). Extracellular superoxide dismutase and its role in cancer. *Free Radical Biology and Medicine*, *112*, 464-479.

Gutierrez, R. M. P., & Navarro, Y. T. G. (2010). Antioxidant and hepatoprotective effects of the methanol extract of the leaves of Satureja macrostema. *Pharmacognosy Magazine*, *6*(22), 125.

Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.

Hangalapura, B. N., Nieuwland, M. G., de Vries Reilingh, G., Heetkamp, M. J., Van den Brand, H., Kemp, B., & Parmentier, H. K. (2003). Effects of cold stress on immune responses and body weight of chicken lines divergently selected for antibody responses to sheep red blood cells. *Poultry Science*, *82*(11), 1692-1700.

Hussain, R., Ali, F., Rafique, A., Ghaffar, A., Jabeen, G., Rafay, M. and Masood, A. 2019. Exposure to Sub-Acute Concentrations of Glyphosate Induce Clinico-Hematological, Serum Biochemical and Genotoxic Damage in Adult Cockerels. Pakistan veterinary journal, 39(2).

Imler, J. L., & Hoffmann, J. A. (2001). Toll receptors in innate immunity. *Trends in cell biology*, *11*(7), 304-311.

Kourouma, A., Quan, C., Duan, P., Qi, S., Yu, T., Wang, Y., & Yang, K. (2015). Bisphenol A induces apoptosis in liver cells through induction of ROS. *Advances in Toxicology*, *2015*.

Ladokun, A.O., Yakubu, A., Otire, 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.

Lillehoj, H. S., & Lee, K. W. (2012). Immune modulation of innate immunity as alternatives-to-antibiotics strategies to mitigate the use of drugs in poultry production. *Poultry science*, *91*(6), 1286-1291.

Marks, H. L. (1985). Direct and correlated responses to selection for growth. *Poultry genetics and breeding*, 47-57.

Matsui H, Oyama TM, Okano Y, Hashimoto E, Kawanai T, Oyama Y (2010) Low micromolar zinc exerts cytotoxic action under H2O2- induced oxidative stress: excessive increase in intracellular Zn2+ concentration. Toxicology 276:27–32

Medzhitov, R., & Janeway Jr, C. A. (1997). Innate immunity: impact on the adaptive immune response. *Current opinion in immunology*, *9*(1), 4-9.

Monckton, V., Ellis, J. L., & Harlander-Matauschek, A. (2020). Floor Substrate Preferences of Chickens: A Meta-Analysis. Frontiers in Veterinary Science, 7, 584162

Mpenda, F. N., Schilling, M. A., Campbell, Z., Mngumi, E. B., & Buza, J. (2019). The genetic diversity of local African chickens: A potential for selection of chickens resistant to viral infections. *Journal of Applied Poultry Research*, *28*(1), 1-12.

Msoffe, P. L. M., Mtambo, M. M. A., Minga, U. M., Gwakisa, P. S., Mdegela, R. H., & Olsen, J. E. (2002). Productivity and natural disease resistance potential of free-ranging local chicken ecotypes in Tanzania. *Livestock Research for rural development*, *14*(3), 2002.

Natt, M. P., & Herrick, C. A. (1952). A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poultry Science, 31(4), 735-738..

Nazıroğlu M, Yıldız K, Tamtürk B, Erturan İ, Flores-Arce M (2012) Selenium and psoriasis. Biol Trace Elem Res 150(1–3):3–9

Obeten, K. E., Uruakpa, K. C., & Isaac, V. (2013). The evaluation of the effect of Sida acuta leaf extract on the microanatomy and some biochemical parameters on the liver of Wistar rats. *Group*, *41*, 2-71

Parish, C. R., & O'neill, E. R. (1997). Dependence of the adaptive immune response on innate immunity: some questions answered but new paradoxes emerge. *Immunology and cell biology*, *75*(6), 523-527.

Pértille, F., Ibelli, A. M. G., Sharif, M. E., Poleti, M. D., Fröhlich, A. S., Rezaei, S., ... & Coutinho, L. L. (2020). Putative epigenetic biomarkers of stress in red blood cells of chickens reared across different biomes. *Frontiers in genetics*, *11*, 508809.

Qureshi, M. A., & Havenstein, G. B. (1994). A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed “typical” 1957 and

1991 broiler diets. *Poultry science*, *73*(12), 1805-1812.

Romero, L. M., & Reed, J. M. (2005). Collecting baseline corticosterone samples in the field: is under 3 min good enough?. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *140*(1), 73-79.

Saleh, M. A., Al-Salahy, M. B., & Sanousi, S. A. (2009). Oxidative stress in blood of camels (Camelus dromedaries) naturally infected with Trypanosoma evansi. *Veterinary parasitology*, *162*(3-4), 192-199.

Shapiro, F., Nir, I., & Heller, D. (1998). Stunting syndrome in broilers: effect of stunting syndrome inoculum obtained from stunting syndrome affected broilers, on broilers, leghorns and turkey poults. *Poultry Science*, *77*(2), 230-236

Siegel, P. B., Katanbaf, M. N., Anthony, N. B., Jones, D. E., Martin, A., Gross, W. B., & Dunnington, E. A. (1987). Responses of chickens to Streptococcus faecalis: genotype-housing interactions. *Avian Diseases*, 804-808.

Stevenson, R. D., Tuberty, S. R., Defur, P. L., & Wingfield, J. C. (2005). Ecophysiology and conservation: the contribution of endocrinology and immunology–introduction to the symposium. *Integrative and Comparative Biology*, *45*(1), 1-3.

Szabo, A., Mezes, M., Horn, P., Sütő, Z., Bázár, G. Y., & Romvari, R. (2005). Developmental dynamics of some blood biochemical parameters in the growing turkey (Meleagris gallopavo). *Acta Veterinaria Hungarica*, *53*(4), 397-409.

Xu, J. X., Cao, C. Y., Sun, Y. C., Wang, L. L., Li, N., Xu, S. W., & Li, J. L. (2014). Effects on liver hydrogen peroxide metabolism induced by dietary selenium deficiency or excess in chickens. *Biological trace element research*, *159*(1), 174-182.

Zarnab, S., Chaudhary, M. S., Javed, M. T., Khatoon, A., Saleemi, M. K., Ahmed, T. and Peng, Y. (2019). Effects of Induced High Ammonia Concentration in Air on Gross and Histopathology of Different Body Organs in Experimental Broiler Birds and its Amelioration by Different Modifiers. Pakistan veterinary journal, 39(3).