



HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF BROILER CHICKS FED DIFFERENT INCLUSION LEVELS OF GINGER (*Zingiber officinale*) AND GARLIC (*Allium sativum*) OIL MIXTURE

R. A. OLUWAFEMI¹, DANIEL, STEPHEN ESSIEN¹ AND J. O. ALAGBE^{2*}

¹Department of Animal Science, University of Abuja, Gwagwalada, Nigeria.

²Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Received: 30 July 2021

Accepted: 06 October 2021

Published: 09 October 2021

Original Research Article

ABSTRACT

The objective of the present study was to determine the effect of dietary inclusion of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) oil mixture (GGOM) on some haematological and serum biochemical indices of broiler chicken. A total of one-day-old broiler chicks (Ross 308) of mixed sex were randomly divided into five treatments with three replicates consisting of 10 birds each in a completely randomized design. Birds in treatment 1 (T1) were fed basal diet with 0 % GGOM while T2, T3, T4 and T5 were given GGOM at 0.1 %, 0.2 %, 0.3 %, and 0.4 % respectively. The experiment lasted for 8 weeks; food and water were provided *ad libitum* and all other management were strictly observed. Data collected were used to evaluate some haematological and serum biochemical indices of animals. Haematological parameters cover pack cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC) and its differentials while serum analysis includes total protein (Tp), globulin, albumin, aspartate serum aminotransferase (AST), alanine amino transferase (ALT) and alanine phosphatase (ALP). All the haematological parameters were significantly ($P < 0.05$) different among the treatment. Similarly, serum biochemical parameters were significantly ($P < 0.05$) affected by GGOM. Increasing the level of GGOM from 0.1 % to 0.4 % tended to reduce ALP, AST, and ALT values. It was concluded that feeding birds at 0.4 % did not cause any negative effect on the health of the animals; the data revealed that all values were within the physiological reference range for broiler chicks.

Keywords: Broilers; ginger; garlic; haematology; serum indices.

1. INTRODUCTION

Nutrition or feeding is one of the major key areas of livestock management that influences the response of poultry to a disease challenge. According to Bamishaiye et al. [1], the nutritional status of an

animal is independent of dietary intake and effectiveness of metabolic processes which can be determined by accessing the blood. Blood is a vital special circulatory tissue is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis

*Corresponding author: Email: dralagbe@finmail.com;

[2]. It is also an important index of physiological and pathological changes in animals [3]. Blood parameters (haematology and serum biochemical indices) are influenced by the quality and quantity of feed, antinutritional factors as well as phytochemicals in the feed [4].

Haematological components consist of red blood cells, white blood cells or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of farm animals [5]. Haematological and serum biochemical analysis has been found to be useful in the diagnosis of several diseases, feed quality as well as investigation to the extent of damage to blood (Khan and Zafar, 2005; Ovuru and Ekweozor, 2004). Red blood cell is involved in the transport of oxygen and carbon dioxide in the body while defending the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response [6]. Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates [7].

The use of essential oil from ginger, garlic, turmeric, clove, and other plants with pathogenic properties has been reported to have a significant influence on the blood parameters of animals. For instance, capsicum and turmeric oils provide protective immunity against diseases by increasing the population of white blood cells to produce more antibodies in the body of animals (Lee *et al.*, 2013). Supplementation of garlic oil at 10 mg/kg significantly increases body weight and serum antibody titers against pathogenic infection [8]. Combinations of oils exert a synergistic effect to reduce negative consequences of pathogenic bacteria (Lee *et al.*, 2013). Essential oils (EOs) have been reported to be safe, effective and could be used to bridge the gap between food safety and animal production [9]. Among the potential EOs is garlic and ginger which are found to be rich in several nutrients, amino acid, minerals and secondary metabolites (bioactive chemicals). Secondary metabolites from ginger and garlic enable them to perform multiple biological activities such as: antimicrobial, antioxidant, anti-inflammatory, antiviral, antifungal, etc. [9].

In view of this potential in ginger and garlic oil, this experiment was designed to examine the effect of dietary inclusion of ginger and garlic oil mixture on the haematology and serum biochemical indices of broiler chicks.

2. MATERIALS AND METHODS

2.1 Site of the Experiment

This study was carried out at the Department of Animal Science, University of Abuja Teaching and Research Farm, Main Campus, along airport Road, Gwagwalada, Abuja, Nigeria between the months of January to March, 2021.

2.2 Identification and Extraction of Ginger and Garlic Oil (GGOM)

Fresh samples of ginger and garlic rhizomes were purchased from a local market in Gwagwalada Abuja, Nigeria. The samples were sorted out of the bad ones, then washed and peel manually with a kitchen knife to remove the outer covering of the rhizomes. It was shade dried separately for 14 days to retain the bioactive chemicals in the plants and milled into powder using a laboratory blender (Panasonic: Model 07A-08C). Extraction of the oil was done using the Soxhlet extraction method. The solvent used is petroleum ether and adjusted to 65°C to reach a vaporization point before the filtrate was exposed to the atmosphere and the residual solvent was allowed to evaporate before extracting the oil. The extracted oil was extracted separately and mixed in a ratio of 1: 1 to obtain ginger and garlic oil mixture (GGOM). The sample obtained was stored in a well-labeled container for further analysis.

2.3 Animal Management

Two hundred one-day-old (Ross 307) broiler chicks of mixed sex were used for the experiment. The birds were purchased from a commercial hatchery in Ibadan, Oyo State, Nigeria. Prior to the arrival of the birds, experimental cages were properly fumigated two weeks before the study, surroundings were cleaned and a foot bath was made available to ensure strict biosecurity. Feeding and water troughs were properly washed and each treatment was labeled for easy identification. Birds were weighed on arrival on the farm to obtain their initial body weight and given anti-stress to reduce stress and prevent mortality. A battery cage housing system was used for the experiment and birds were divided into five treatments with 4 replicates consisting of ten birds each in a completely randomized design. 200 watts bulbs were used as a source of heat and newspapers were laid on the floor of the cage to allow easy access to the movement of the birds. All other management practices were strictly observed throughout the experiment which lasted for 56 weeks.

2.4 Feed Formulation

Three basal diets were formulated at different stages of production to meet up the requirements of birds according to NRC (1994) as presented in Table 1. Broiler starter's mash (1-21 days), Growers mash (22-35 days), and finishers mash (36-56 days).

2.5 Experimental Set-up

Treatment 1: Basal diet + 0 % GGOM
 Treatment 2: Basal diet + 0.2 % GGOM
 Treatment 3: Basal diet + 0.3 % GGOM
 Treatment 4: Basal diet + 0.4 % GGOM
 Treatment 5: Basal diet + 0.5 % GGOM

2.6 Data Collection

Feed intake (g) was determined by subtracting feed leftover from feed served, it was estimated for each of the replicates daily.

Weight gain (g) = final weight – initial weight

2.7 Blood Collection

At the end of the experiment blood samples were collected very early in the morning (8:00 am) from three (3) randomly selected birds per replicate for haematological and serum analysis, the animals were not stressed to prevent oxygenated blood from becoming deoxygenated. About 5 ml of the sample was emptied into a labeled ethylene diamine tetraacetate (EDTA) bottle for haematological analysis. Pack cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and its differentials (lymphocytes, neutrophils, and eosinophils) were determined according to the methods outlined by Coles (1986). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as:

$$\text{MCV (fl)} = \text{PCV} \times 10 / \text{RBC mm}^3$$

$$\text{MCH (pg)} = \text{Hb in g/100 ml blood} \times 10 / \text{RBC mm}^3$$

$$\text{MCHC (\%)} = \text{Hb in g/100 ml blood} \times 10 / \text{PCV}$$

Blood samples for serum biochemistry were collected into bottles without EDTA. Total protein, albumin, globulin, aspartate serum aminotransferase (AST), alanine amino transferase (ALT) and alanine phosphatase (ALP) were determined using commercial test kits (GST- 4509 A model, Punjab, India).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Phytochemical analysis for the evaluation of bioactive compounds in GGOM was carried out using a gas chromatography-mass spectrophotometer (Model GR-0TR, Yanyung, China).

3.2 Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) using SPSS version 22 and significant means were separated using the software of the same package. A significant difference was declared if $P \leq 0.05$. The model used in this experiment is:

$$T_{xy} = \mu + \alpha x + \beta xy$$

Where T_{xy} = response variables; X = overall mean; αx = effects on treatments and βxy = random error.

3.3 Bioactive Compounds in GGOM

Bioactive compounds in GGOM are presented in Table 2. The sample contains allicin (48.71 %), zingiber (40.19 %), β -myrcene (7.22 %), β -phellandrene (0.93 %), α -terpinene (1.00 %), α -phellandrene (1.00 %) and α -humulene (0.95 %) respectively. Allicin had the highest concentration followed by zingiber, β -myrcene, α -terpinene, α -phellandrene and β -phellandrene in order of abundance. Bioactive chemicals are secondary metabolites or phytochemicals which makes plant perform several pharmacological activities such as antibacterial, antifungal, antioxidants, hypolipidemic, hepatoprotective, antimicrobial and antiviral [10-12]. Their concentrations vary from one specie to another and other factors such as method of extraction, geographical location, antinutrients, harvesting season, storage conditions and part of plants used (Hyun *et al.*, 2018) [13]. They are also generally regarded as effective and safe [14]. For instance, flavonoids have been shown to perform anti-inflammatory, antiallergic, antithrombotic and antibacterial activities (Iniaghe *et al.*, 2009). They are also used as adjuvants during the production of vaccine [15]. Phenols are antioxidants that are capable of scavenging free radicals or reactive oxygen species, thus preventing diseases in the body (Adesuyi *et al.*, 2012). Tannins are known to perform antibacterial, antiviral and antitumor activities (Tijjani *et al.*, 2012).

Table 1. Chemical composition of experimental diets

Materials	Starter (1-21 days)	Grower (22-35 days)	Finisher (36-56 days)
Maize	50.00	55.00	60.00
Wheat offal	8.00	8.00	8.05
Soya meal	28.55	22.00	21.00
Groundnut cake	10.00	11.55	6.05
Fish meal	2.00	2.00	2.00
Bone meal	0.35	0.40	0.40
Limestone	0.20	0.20	0.20
Lysine	0.15	0.15	0.15
Methionine	0.20	0.20	0.20
Premix	0.25	0.25	0.25
Salt	0.30	0.30	0.30
TOTAL	100.0	100.0	100.0
Calculated analysis			
Crude protein	23.08	20.11	19.33
Ether extract	5.03	4.87	4.28
Crude fibre	3.06	3.95	3.42
Calcium	0.98	1.00	1.10
Phosphorus	0.47	0.40	0.51
Lysine	1.17	1.29	1.60
Meth +Cyst	0.87	0.82	0.51
ME (Kcal/kg)	2936	3000.8	3100.2

*Premix supplied per kg diet: - vit A, 13,000 I.U; vit E, 5mg; vit D3, 3000I.U, vit K, 3mg; vit B2, 5.5mg; Niacin, 25mg; vit B12, 16mg; choline chloride, 120mg; Mn, 5.2mg; Zn, 25mg; Cu, 2.6g; folic acid, 2mg; Fe, 5g; pantothenic acid, 10mg; biotin, 30.5g; antioxidant, 56mg.

Table 2. Bioactive compounds in GGOM

Parameters	% Composition
Allicin	48.71
Zingiber	40.19
β-myrcene	7.22
β-phellandrene	0.93
α-terpinene	1.00
α- phellandrene	1.00
α-humulene	0.95

3.4 Effect of Dietary Inclusion of Ginger and Garlic Oil Mixture on the Haematological Parameters of Broiler Chicks

Effect of dietary inclusion of ginger and garlic oil mixture on the haematological parameters of broiler chicks is presented in Table 3. Pack cell volume value range between 26.20 – 32.66 %, red blood cell 7.92 – 11.80 (10¹²/L), haemoglobin 8.26 – 16.92 g/dL, mean corpuscular haemoglobin (56.60 – 75.03 fl), mean corpuscular volume (34.80 – 69.59 pg), mean corpuscular haemoglobin concentration (61.09 – 89.22 %), white blood cell 20.80 – 32.53 (10⁹/L), eosinophils (0.94 – 1.21 %), monocytes (1.09 – 1.82 %), basophils (1.40 – 2.13 %) and lymphocytes (10.04 – 19.17 %) respectively. All the results were obtained to follow the trend and were highest in T3, T4, and T5, intermediate in T2, and lowest in T1 (P<0.05).

The values were significantly (P<0.05) different among the treatments. However, they were within the normal physiological range for birds reported by Talebi *et al.* (2005;) [16] a deviation from the normal range is could be a result of nutritional, environmental, and pathological factors (Olajide and Akinleye, 2010). Blood components perform different functions in the body of animals, for instance, red blood cells are responsible for the transportation of oxygen, carbon dioxide, and other nutrients in the body [2]. A low level of PCV is a clear indication of anaemia [17]. PCV, RBC and Hb range (26.20 – 32.66 %), 7.92 – 11.80 (10¹²/L), and 8.26 – 16.92 g/dL is within the range reported by Forlan *et al.* [18]. Haemoglobin aids in the transport of oxygen to the tissues of the animal for oxidation of ingested food so as to release energy [6]. Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration indicate blood level conditions [19]. WBC plays a key role in the production of antibodies, thus strengthening the immune system of animals. Therefore high WBC values in T4 and T5 is an indication that the birds have high resistance to diseases [20]. Leucocytes are sensitive biomarkers crucial to immune function [21]. The variations in the haematological parameters could be attributed to the presence of phytochemicals in GGOM. The result obtained in this study is in agreement with the findings of Kim *et al.* [8] who reported that

Table 3. Effect of dietary inclusion of ginger and garlic oil mixture on the haematological parameters of broiler chicks

Parameters	T1	T2	T3	T4	T5	SEM
PCV (%)	26.20 ^b	28.07 ^b	30.60 ^a	31.06 ^a	32.66 ^a	0.49
RBC ($\times 10^{12}/L$)	7.92 ^c	9.03 ^b	10.80 ^b	11.22 ^a	11.80 ^a	3.07
Haemoglobin(g/dL)	8.26 ^c	10.42 ^b	15.60 ^b	16.80 ^a	16.92 ^a	2.21
MCH (fl)	56.60 ^c	65.11 ^b	71.06 ^a	73.44 ^a	75.03 ^a	4.35
MCV(pg)	34.80 ^c	41.02 ^b	59.7 ^a	61.91 ^a	69.59 ^a	1.62
MCHC (%)	61.09 ^c	72.42 ^b	83.08 ^a	86.42 ^a	89.22 ^a	2.05
WBC ($\times 10^9/L$)	20.80 ^c	28.92 ^b	31.09 ^a	31.47 ^a	32.53 ^a	0.77
Eosinophils (%)	0.94 ^c	1.02 ^a	1.07 ^a	1.15 ^a	1.21 ^a	0.10
Monocyte (%)	1.09 ^b	1.20 ^b	1.26 ^b	1.39 ^b	1.82 ^a	0.06
Basophils (%)	1.40 ^b	1.80 ^b	1.86 ^b	2.01 ^a	2.13 ^a	0.03
Lymphocytes (%)	12.04 ^c	15.03 ^b	15.11 ^b	18.15 ^a	19.17 ^a	1.69

Mean in the same row with different superscripts are significantly different ($P < 0.05$).

MCH: mean corpuscular haemoglobin; MCV: mean corpuscular volume; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cell; Eosinophil; Monocyte; Basophils; Lymphocytes.

Table 4. Effect of dietary inclusion of GGOM on serum biochemical indices of broiler chicks

Parameters	T1	T2	T3	T4	T5	SEM
Alb (g/dL)	2.04 ^b	2.07 ^b	2.11 ^a	2.25 ^a	2.38 ^a	0.01
GLO(g/dL)	1.92 ^b	2.11 ^a	2.30 ^a	2.32 ^a	2.41 ^a	0.02
TP(g/dl)	3.96 ^b	4.18 ^a	4.41 ^a	4.57 ^a	4.79 ^a	0.14
ALP (μ/L)	62.42 ^a	50.69 ^a	41.62 ^b	40.88 ^b	38.80 ^c	0.90
ASP(μ/L)	98.16 ^a	84.20 ^a	60.61 ^b	51.50 ^b	48.72 ^c	1.58
ALT(μ/L)	54.46 ^a	50.56 ^a	40.82 ^b	39.90 ^b	34.33 ^c	0.88

Means in the same row with different superscripts are significantly different ($P < 0.05$)

Alb: Albumin; GLO: globulin; TP: total protein; ALP: alanine phosphatase; ASP: alanine serum phosphatase; ALT: alanine transaminase

supplementation of 10 mg/kg of garlic in broiler chick's increased protective immunity against pathogenic organisms. The combination of multiple phytochemicals exerts a synergistic effect to scavenge free radicals [22].

Table 4 Effect of dietary inclusion of ginger and garlic oil mixture on serum biochemical parameters of broilers chick.

Table 4 reveals the effect of dietary inclusion of ginger and garlic oil mixture on serum biochemical parameters of broilers chicks. Albumin (Alb), globulin (GLO), total protein (TP), amino serum transferase (AST), alanine serum phosphatase (ASP) and alanine transaminase (ALT) values ranged between 2.04 – 2.38 g/dL, 1.92 – 2.41 g/dL, 3.96 – 4.57 g/dL, 38.80 – 62.42 (μ/L), 48.72 – 98.16 (μ/L) and 39.90 – 54.46 (μ/L) respectively. TP values were highest in T2, T3, T4 and T5, lowest in T1 ($P < 0.05$). ALP, AST and ALP values were highest in T1 and T2, intermediate in T3 and T4, lowest in T5 ($P < 0.05$). All the serum biochemical indices were significantly ($P < 0.05$) different among the treatments. Higher TP recorded in T5 is an indication that the animals were well nourished due to the presence of nutrients in

GGOM. Protein reserve across the treatment is enough to support the growth and general performance of the animal. The outcome of this result is in agreement with the findings of Singh *et al.* (2012). ALT, AST and ALP values decreased as the level of GGOM inclusion increases in the diet. This result agrees with the findings of Alagbe *et al.* [23] who reported a decrease in serum enzymes of rabbits fed *Albizia lebbbeck* oil. However, the values reported are within the normal physiological range for healthy rabbits reported by Özkan *et al.* [24]. According to Alagbe [23] serum enzymes values are triggered by the presence of antinutrients or toxic substances in the feed of an animal. ALT, AST and ALP values recorded in this experiment decreases as the level of GGOM inclusion increases across the diets ($P < 0.05$). This is a clear indication that the test material (GGOM) is within the tolerable limit for the animals.

4. CONCLUSION

It was concluded from this experiment that GGOM is rich in several bioactive chemicals which are safe, effective, and could be used as an alternative to antibiotics. Its inclusion at 0.4 % in the diet of broiler

chicks had no negative effect on all the blood parameters examined.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bamishaiye EI, Muhammad NO, Bamishaiye OM. Haematological parameters of albino rats fed on tiger nuts (*Cyperus esculentus*) tuber oil meal-based diet. The International Journal of Nutrition and Wellness. 2009;10(1). Available: <http://ispub.com/IJNW/10/1/9293>
2. Isaac LJ, Abah G, Akpan B, Ekaette IU. Haematological properties of different breeds and sexes of rabbits. Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria. 2013;24-27.
3. Mitruka BM, Rawnsley HM. Clinical biochemical and hematological reference values in normal experimental animals. USA: Masson Publishing Inc. 1977;134-135.
4. Akinmutimi AH. Evaluation of Sword bean as an alternative feed resources for broiler chicken. PhD thesis, Michael Okpara University of Agriculture, Umudike, Nigeria; 2004.
5. Oyawoye BM, Ogunkunle HN. Biochemical and haematological reference values in normal experimental animals. New York: Masson. 2004;212-218.
6. Soetan KO, Akinrinde AS, Ajibade TO. Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (*Sorghum bicolor*). Proceedings of 38th Annual Conference of Nigerian Society for Animal Production. 2013;49-52.
7. Maton A, Hopkins RLJ, McLaughlin CW, Johnson S, Warner CW, LaHart D, Wright JD. Human biology and health. Englewood Cliffs, New Jersey, USA: Prentice Hall; 1993.
8. Kim DK, Lillehoj HS, Lee SH, Lillehoj EP, Bravo D. Improved resistance to *Eimeria* spp in chicken due to dietary supplementation with garlic metabolites. Brazilian Journal of Nutrition. 2013;109:76-88.
9. Olafadehan OA, Oluwafemi RA, Alagbe JO. Performance, haemato-biochemical parameters of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. Advances in Research and Reviews. 2020;1:4.
10. Singh AS, Alagbe JO, Sharma S, Oluwafemi RA, Agubosi OCP. Effect of dietary supplementation of melon (*Citrallus linatus*) seed oil on the growth performance and antioxidant status of growing rabbits. Journal of Multidimensional Research and Reviews. 2021;2(1):78-95.
11. Adewale AO, Alagbe JO, Adeoye, Adekemi Grace. Dietary supplementation of rauwolfia vomitoria root extract as a phyto-genic feed additive in growing rabbit diets: haematology and serum biochemical indices. Scopia International Journal for Science, Commerce and Arts. 2021;1(3):16-27.
12. Musa B, Alagbe JO, Adegbite Motunrade Betty, Omokore EA. Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture. United Journal for Research and Technology. 2020;2(2):13-21.
13. Shittu MD, Alagbe JO. Phyto-nutritional profiles of broom weed (*Sida acuta*) leaf extract. International Journal of Integrated Education. 2020;3(11):119-124.
14. Akintayo Balogun Omolere M, Alagbe JO. Probiotics and medicinal plants in poultry nutrition: A review. United International Journal for Research and Technology. 2020;2(1):7-13.
15. Alagbe JO, Adegbite Motunrade Betty. Haematological and serum biochemical indices of starter broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* bark mixture. International Journal of Biological, Physical and Chemical Studies. 2019;1(1):8-15.
16. Oguz H, Kececi T, Birdane YO, Önder F, Kurtoglu V. Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Research in Veterinary Science. 2000;69(1):89-93.
17. Ugwuene MC. Effect of dietary palm kernel meal for maize on the haematological and serum chemistry of broiler Turkey. Nigerian Journal of Animal Science. 2011;13:93-103.
18. Forlan RL, Macari M, Malheiros RD, Moraes VMB, Malheiros EB. Effect of age and strain on haematology and blood-biochemical parameters in broiler chickens. Revista Brasileira de Ciencia Avicola. 1999;1(2):117-122.
19. Chineke CA, Ologun AG, Ikeobi CON. Haematological parameters in rabbit breeds and crosses in humid tropics. Pakistan Journal of Biological Sciences. 2006;9(11):2102-2106.
20. Aster JC. Anaemia of diminished erythropoiesis. In V. Kumar, A. K. Abbas, N. Fausto, S. L. Robbins, & R. S. Cotran (Eds.), Robbins and Cotran Pathologic Basis of

- Disease (7th ed, p.638-649). Saunders Co. Philadelphia; 2004.
21. Shaniko S. Physiological responses of laying hens to the alternative housing systems. *Int. J. Poultr. Sci.* 2003;2:357-360.
 22. Kim DK, Lillehoj HS, Lee SH, Jang SI, Bravo D. High through put gene expression analysis of intestinal intraepithelial lymphocytes after oral feeding of carvacrol, cinnamaldehyde or *Capsicum oleoresin*. *Journal of Poultry Science.* 2010;89:68-81.
 23. Alagbe JO. Proximate, mineral and phytochemical analysis of *Piliostigma thonningii* stems bark and roots. *International Journal of Biological, Physical and Chemical Studies.* 2019;1(1):1-7.
 24. Özkan C, Kaya A, Akgül Y. Normal values of haematological and some biochemical parameters in serum and urine of New Zealand White rabbits. *World Rabbit Science.* 2012; 20(4):253-259.