

## Research Article

### Effect of Aqueous *Piliostigma Thonningii* Leaf Extracts on the Hematological and Serum Biochemical Indices of Broiler Starter Chicks

Alagbe John<sup>1</sup>✉, Sharma DO<sup>1</sup> and Xing Liu<sup>2</sup>

<sup>1</sup>Department of Animal Production, Sumitra Research Farm, Gujarat, India

<sup>2</sup>Norton Quality Control Laboratory, Norway

#### Abstract

Three hundred one-day-old broiler chicks (Ross 308) were used to evaluate the effect of aqueous *Piliostigma thonningii* Leaf Extracts (PTE) on the some hematological and serum biochemical parameters of broiler chicks. The birds were randomly assigned to five treatments of four replicates consisting of 15 birds each in a completely randomized design. Birds in Treatment 1 (T1) were given PTE at 0 % while T2, T3, T4 and T5 were fed PTE at 20ml, 40ml, 60ml and 80ml per liter of water. Feed and water were offered *ad libitum* throughout the experiment which lasted for 4 weeks. The hematological parameters examined are: Pack Cell Volume (PCV), Hemoglobin (Hb), Red Blood Cells (RBC), Erythrocyte Sedimentation Rate (ESR), White blood cells (WBC) and its differentials while those of serum biochemical parameters are: Albumin, globulin, total protein, cholesterol, creatinine, urea, Serum Glutamic Oxaloacetate (SGOT) and Serum Glutamic Phospho-Transaminase (SGPT). Result obtained showed that all the hematological parameters were analyzed were higher in bird in T5 compared with control group ( $P < 0.05$ ). Albumin, globulin, total protein, cholesterol, urea, SGPT and SGOT values were significantly reduced as the level of PTE increases across the treatments ( $P < 0.05$ ). Creatinine level were not significantly ( $p > 0.05$ ) different among the treatments as an indication that the integrity of the kidney was not compromised. It could be concluded that PTE at levels up to 80 ml have no deleterious effect on the blood profile of birds.

\*Corresponding author: Alagbe John, Department of Animal Production, Sumitra Research Farm, Gujarat, India, Tel: +91 80 66671794; E-mail: demson-farms@yahoo.com

Citation: John A, DO S, Liu X (2020) Effect of Aqueous *Piliostigma Thonningii* Leaf Extracts on the Hematological and Serum Biochemical Indices of Broiler Starter Chicks. J Protein Res Bioinform 2: 006.

Received: March 03, 2020; Accepted: April 09, 2020; Published: April 20, 2020

Copyright: © 2020 John A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Keywords:** Broiler chicks; Hematology; *Piliostigma thonningii* leaf extract

#### Introduction

Plants are natural gift to man because they contain all necessary nutrients and several valuable bioactive compounds [1,2] and also provided a clue on the discovery of new products of medicinal value for drug development. According to [3] out of the about 250,000-500,000 plant species estimated by the [4], only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties.

Among the underexplored leguminous plant is *Piliostigma thonningii* which is found to be loaded in several secondary metabolites such as flavonoids, phenol, tannins and alkaloids [5,6], minerals and vitamins [7]. The plant belongs to the family *Caesalpi niacea* and found in abundant in most part of the world including Africa and Asia. The plant leaves have been reported to contain 10.09 % protein, 2.81 % fat, 6.10 % ash, 5.23 % crude fibre, 72.17 % carbohydrates and also have several biological effects, exhibiting antibacterial [8], anti-inflammatory, anthelmintic, hypocholesterolemic [9], immunomodulatory [10], hypoglycemic [11] and hematopoietic properties [12].

Several studies have been carried out on different plant extracts on animal's performance. According to clove extract at 400 ppm caused a significant ( $P < 0.05$ ) increase in final weight of broilers. *Piliostigma thonningii* leaf extract at 0.4g/kg of body weight has also been reported to reduce blood cholesterol level in Wistar rats. According to [13], *Parkia biglobosa*, *Delonix regia* leaf extract and garlic/ lemon grass extract have helped to improve weight gain, feed conversion ratio, blood profile and reduce mortality in birds. However, limited information is available on the effect of PTE on the blood profile of broilers. A timely evaluation of PTE will give a clue on the immune system of the animal and provides its safe recommended level in broiler chicks.

#### Materials and Methods

##### Site of the experiment

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Farm, Gujarat, India during the month of August to October, 2019.

##### Collection and processing of test material

Fresh and mature leaves (*Piliostigma thonningii*) were obtained from the premises of Sumitra research farm in India and was authenticated by a taxonomist and assigned a voucher number (FS-09-345), air dried and later grinded into fine particles using a laboratory blender. The extract (PTE) was prepared by putting 150 grams of the powdered sample in 1000 ml of distilled water for 2 days after which

the extract was filtered using Whatman filter paper No. 1 and kept in refrigerator at 4°C for further analysis. The powdered sample was also subjected to proximate analysis.

### Experimental animals and management

Three hundred one-day old Ross 308 broiler chicks were used for the experiment. Prior to the commencement of the study, the pens were properly disinfected with Morigad at 10 ml to 50 liters of water, feeders and drinkers were thoroughly washed and all other electrical fittings were fixed. The birds were weighed on arrival to the farm and thereafter weekly and distributed randomly into five (5) treatments of 300 chicks of four replicates each consisting of 15 birds and electric brooders were used as source of heat. All other management practices were strictly observed and a vaccination program was designed, vaccines were administered according to the prevailing disease condition in the environment. Water soluble multi-vitamins (Biovite super® at 1ml to 5 litres of water) was given to the chicks before 3 days of vaccination and 3 days after vaccinations in order to guard stress. Fresh, clean and cool drinking water was provided to the experimental bird's *ad-libitum*.

### Feed formulation and experimental design

Birds were fed basal diet formulated according to NRC. Starter diet was given between (0-4 weeks) containing a crude protein of 23.40 % and metabolizable energy of 2950.6 kcal/ kg. Treatment 1 (Control) was given 0 % PTE, treatment 2, 3, 4 and 5 were given PTE at levels 20 ml, 40ml, 60ml and 80 ml/ liter respectively. The experimental design that was used is a Completely randomized design (CRD).

### Blood analysis

At 4<sup>th</sup> week, twelve birds were randomly from each treatment for haematological and serum biochemical analysis. Selected animals were kept in a stress-free environment to prevent oxygenated blood becoming deoxygenated during blood collection. The sampled birds were bled from punctured wing vein to aspire 5mls of blood from each bird out of which 2mls was collected into bijoux bottle treated with Ethylene Diamine Tetra Acetate (EDTA) for haematological assay. Complete blood analysis was performed within three (3) hours of collection using a commercial diagnostic kit (Nosrac diagnostic analyzer, China). Pack cell volume was estimated by micro haematocrit method [14]. Red blood cell, haemoglobin, white blood cell and its absolute counts were determined by using Neubauer's chamber. Values of MCV, MCH and MCHC were calculated using:

- $MCV (fl) = PCV/RBC \times 100$
- $MCH (pg) = Hb/RBC \times 10$
- $MCHC (\%) = Hb (100mg\ blood)/PCV \times 100$

Blood for serum analysis was collected into bottles free from anticoagulant and taken to the laboratory for further analysis. Commercial diagnostic kits were used for determination of total protein, calcium, sodium, bicarbonate, phosphorus, total cholesterol, low density lipoprotein, high density lipoprotein, glucose, triglycerides, urea, creatinine, total bilirubin, along with the activity of the following enzymes: alanine transaminase, aspartate transaminase, serum glutamic oxaloacetate transaminase and serum glutamic pyruvic transaminase were computed according to the method outlined by [15].

### Laboratory analysis

Proximate analysis of feed (crude protein, crude fiber, ether extracts and ash) were determined in accordance with the Official Methods of the Association of Official Analytical Chemists [16]. Phytochemical analysis of saponins, flavonoids, phenolics, alkaloids, steroids and glycosides using standard methods described by [17,18]. Tannins [19] and flavonoids [20] contents were also determined in the extracts.

### Statistical analysis

All data collected were subjected to one-way analysis of variance (ANOVA) using SPSS (25.0) and significant means was separated using [21] multiple range tests significant will be declared if  $P \leq 0.05$ .

\* 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

Means with same superscript are significantly different ( $P < 0.05$ )

RBC: Red blood cell, WBC: white blood cell, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, MCH: Mean corpuscular hemoglobin, SEM: Standard error of mean

### Results

The ingredients composition of experimental diet is presented in (Table 1). The proximate components of the diets revealed the presence of crude protein (23.40 %), crude fibre (4.02 %), ether extract (5.04 %) and metabolizable energy (2950.6 kcal/kg). However, the experimental diet was formulated to meet the nutritional requirement of birds according to NRC.

Ingredients	Starter (0-4 weeks)
Maize	54
Wheat offal	5
Soya meal	30
Groundnut cake	8
Oyster shell	2
Bone meal	3
Lysine	0.2
Methionine	0.2
*Premix	0.25
Salt	0.3
Toxin binder	0.1
Total	
Determined analysis (% DM)	
Crude protein	23.4
Crude fibre	4.02
Ether extract	5.04
Energy (Kcal/kg)	2950.6

**Table 1:** Ingredient composition of the experimental diets.

Proximate components of *Ptilostigma thonningii* leaves shows that it contained moisture, crude protein, crude fibre, ether extract and ash at 8.79 %, 11.21 %, 14.22 %, 0.31 and 7.22 % respectively as presented in (Table 2).

Parameters	% Composition
Moisture	8.79
Crude protein	11.21
Crude fibre	14.22
Ether extract	0.31
Ash	7.22

**Table 2:** Proximate composition of *Piliostigma thonningii* leavess.

Table 3 shows the phytochemical constituents of *Pilio tigma thonningii* leaf extract. The extract revealed the presence of bioactive chemicals like alkaloids, saponins, flavonoids, tannins and steroids. The values obtained are 0.40, 4.38, 9.77, 1.67 and 0.02 (mg/100g) for alkaloids, saponins, tannins, flavonoids and steroids respectively.

Phytochemicals	Composition (mg/100g)	Permissible range
Alkaloids	0.4	3.5
Saponins	4.38	7.02
Tannins	9.77	31.5
Flavonoids	1.67	6.11
Steroids	0.02	1.3

**Table 3:** Phytochemical composition of *Piliostigma thonningii* leaf extract.

Hematological parameters of broiler chicks give different levels of PTE is presented in (Table 4). The parameters determined were Pck cell volume (PCV), Hemoglobin (Hb), Erythrocyte sedimentation rate (ESR), Red blood cell (RBC), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and White blood cell (WBC) with its differentials.

Parameters	T1	T2	T3	T4	T5	SEM
PCV (%)	27.10 <sup>b</sup>	28.57 <sup>b</sup>	32.04 <sup>a</sup>	33.32 <sup>a</sup>	34.80 <sup>a</sup>	1.2
Hb (g/dl)	9.12 <sup>c</sup>	10.29 <sup>b</sup>	11.40 <sup>b</sup>	13.21 <sup>a</sup>	13.60 <sup>a</sup>	0.33
ESR (mm/hr)	1.97 <sup>c</sup>	2.14 <sup>b</sup>	2.87 <sup>b</sup>	3.47 <sup>a</sup>	3.79 <sup>a</sup>	1.44
RBC ( $\times 106/\mu\text{l}$ )	1.93 <sup>b</sup>	2.03 <sup>a</sup>	2.51 <sup>a</sup>	2.88 <sup>a</sup>	2.92 <sup>a</sup>	1.01
MCV (fl)	140.4 <sup>a</sup>	140.7 <sup>a</sup>	127.6 <sup>b</sup>	115.7 <sup>c</sup>	119.2 <sup>c</sup>	1.65
MCH (pg)	47.25	50.69	45.42	45.87	46.58	2.85
MCHC (%)	33.65	36.02	35.58	39.65	39.08	1.1
WBC ( $\times 106/\mu\text{l}$ )	18.73 <sup>a</sup>	21.04 <sup>b</sup>	23.56 <sup>b</sup>	24.70 <sup>b</sup>	25.32 <sup>a</sup>	1.05
Lymphocytes (%)	5.02 <sup>c</sup>	9.13 <sup>b</sup>	9.68 <sup>b</sup>	10.21 <sup>a</sup>	10.45 <sup>a</sup>	0.25
Monocytes (%)	0.6	0.71	0.78	0.71	0.73	0.01
Heterophils (%)	4.50 <sup>c</sup>	5.11 <sup>c</sup>	6.05 <sup>b</sup>	7.18 <sup>a</sup>	7.80 <sup>a</sup>	0.03
Basophils (%)	1.27	1.99	2.07	2.1	2.2	0.02
Eosinophils (%)	0.58	0.66	0.64	0.67	0.61	0.04

**Table 4:** Hematological parameters of broiler chicks fed different levels of PTE.

PCV values obtained are 27.10 %, 28.57 %, 32.04 %, 33.32 % and 34.80 % for T1, T2, T3, T4 and T5 respectively while Hb values are 9.12, 10.29, 11.40, 13.21 and 13.60 (g/dl) for T1, T2, T3, T4 and T5. ESR values are 1.97, 2.14, 2.87, 3.47 and 3.79 (mm/hr) for T1, T2, T3, T4 and T5 respectively. PCV, Hb, ESR, RBC, MCV and WBC values were significantly ( $p < 0.05$ ) different among the treatments. Values for MCH are 47.25, 50.69, 45.42, 45.87 and 46.58 (pg) for T1, T2, T3, T4 and T5 respectively while those of MCHC are 33.65,

36.02, 35.58, 39.65 and 39.08 (%) for T1, T2, T3, T4 and T5. MCH, MCHC, monocytes, basophils and eosinophils were not significantly ( $p > 0.05$ ) influenced by the oral inclusion of PTE.

Table 5 shows the serum biochemical indices of broiler chicks given different levels of PTE. Albumin values obtained are 1.55, 1.71, 1.82, 1.76 and 1.83 for T1, T2, T3, T4 and T5 respectively while those of globulin are 1.00, 1.91, 1.98, 2.00 and 2.03 for T1, T2, T3, T4 and T5 respectively. Values obtained for creatinine 0.63, 0.60, 0.61, 0.66 and 0.64 (mg/dl) for T1, T2, T3, T4 and T5 respectively.

Parameters	T1	T2	T3	T4	T5	SEM
Albumin (g/dl)	1.55	1.71	1.82	1.76	1.83	0.04
Globulin (g/dl)	1.00 <sup>c</sup>	1.91 <sup>b</sup>	1.98 <sup>b</sup>	2.00 <sup>a</sup>	2.03 <sup>a</sup>	1.01
Total protein (g/dl)	2.55 <sup>b</sup>	3.63 <sup>a</sup>	3.70 <sup>a</sup>	3.76 <sup>a</sup>	3.86 <sup>a</sup>	0.09
Creatinine (mg/dl)	0.63	0.6	0.61	0.66	0.64	0.02
Cholesterol (mg/dl)	95.18 <sup>a</sup>	67.08 <sup>b</sup>	66.10 <sup>b</sup>	45.78 <sup>c</sup>	40.90 <sup>c</sup>	3.07
Urea (mg/dl)	1.88 <sup>a</sup>	1.71 <sup>a</sup>	1.45 <sup>b</sup>	1.20 <sup>c</sup>	1.10 <sup>c</sup>	0.04
SGPT (iu/l)	123.9 <sup>a</sup>	120.1 <sup>a</sup>	100.7 <sup>b</sup>	98.03 <sup>c</sup>	80.65 <sup>c</sup>	1.35
SGOT (iu/l)	75.12 <sup>a</sup>	74.30 <sup>a</sup>	59.81 <sup>b</sup>	50.44 <sup>b</sup>	45.56 <sup>c</sup>	1.5

**Table 5:** Serum biochemical indices of broiler chicks fed different levels of PTE.

Total protein, cholesterol, urea, SGPT and SGOT values were significantly ( $p < 0.05$ ) different among the treatments. Urea values obtained are 1.88, 1.71, 1.45, 1.20 and 1.10 (mg/dl) for T1, T2, T3, T4 and T5 while those of cholesterol are 95.18, 67.08, 66.10, 45.78 and 40.90 (mg/dl) for T1, T2, T3, T4 and T5 respectively. No significant difference ( $p > 0.05$ ) was observed in the creatinine values among the treatments.

Means with same superscript are significantly different ( $p < 0.05$ )

Serum glutamic oxaloacetate (SGOT), Serum glutamic phospho-transaminase (SGPT), SEM: Standard error of mean

## Discussion

The crude protein (11.21 %), crude fibre (14.22 %), ash (7.22 %) obtained for *Piliostigma thonningii* leaves in this experiment were higher than those reported by [22]. This differences in the proximate components could be attributed to differences in soil type, age, species, season and location [23]. Phytochemical constituents of *Piliostigma thonningii* leaf extract also reveals that tannin has the highest concentration, followed by saponin, flavonoids, alkaloids and steroids respectively. This composition agrees with the findings of [24] but contrary with reports of who reported a concentration of saponin is highest in ethanolic extraction of *Piliostigma thonningii* leaves. According to [25], extraction method and age of plants are some cardinals that could influence the phytochemical composition of plants. However, all the values reported in this experiment are below the lethal dose reported by [26,27]. According to [28] each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. Phytochemicals are also bioactive chemicals or secondary metabolites which performs multiple biological activities such as antioxidant, anti-microbial effect, modulation of detoxification enzymes, stimulation of the immune system [29]. Tannins have been reported to perform antibacterial and antiviral activities [30].

According to [31] blood are significant tools of accessing the clinical and nutritional health status of animals. Hemato-biochemical analysis has been found useful for disease prognosis and feed stress monitoring [32] and their variations in animals have been attributed to age and sex [33], breed [34], feed replacement [35] and environment. RBC, Hb, PCV, ESR, WBC and MCHC values significantly ( $P < 0.05$ ) increased from treatment 1 to 5. Though the hemoglobin range (9.12 – 13.60 g/dl) reported in this experiment which is slightly higher than 8.00 – 9.60 g/dl recorded by [36], however, all values were within the reference range for broilers [37,38]. According to hematological values cannot be absolutely constant due to variations in age, breed, strain and sampling techniques. An increase in the RBC level is an indication of increased oxygen in the animal tissue [39,40] also opined that a significant increase in PCV reveals a better transport of oxygen and absorbed nutrients, thus result in an increased primary and secondary polycythemia. PCV and MCH are also significant indices in the diagnosis of anemia and also serve as useful parameters of the bone marrow capacity to produce red blood cells in animals [41].

White blood cells play a vital role in the prevention of disease or infection, thus animals with low WBC level stand a risk of disease infections. Animals in  $T_4$  and  $T_5$  with high WBC are capable of generating antibodies and have a high degree of resistance to diseases [42]. Leucocyte count and its differentials have also been reported to increase during stress and unfavorable conditions [43,44]. Described basophils and eosinophils as important effector cells in allergy and host defense responses particularly against parasitic infections.

Total protein, cholesterol, urea, SGPT and SGOT were significantly ( $p < 0.05$ ) different among the treatment, the total protein level increased significantly from treatment 1 to 5. This is a clear indication that the protein reserve in the diet is enough to maintain and support the growth of the animal. According to [45] fluctuation in serum albumin could be attributed to the quality of diet as well as the presence of diseases. Similarly, globulins play a significant role in fighting infections, hormone carrier as well as blood clotting process because of the presence of antibodies and enzymes in them. Cholesterol and urea level decreased as the level of PTE increased. The trend in the cholesterol level is a sign that the meat is safe for consumption and reduces the risk of cardiovascular disease, it also shows that PTE contains hypolipidemic substances. This result agrees with the findings of [46,47] when banana leaf was used as a phytoadditive in the diets of broiler chicks.

Urea is a useful indicator of kidney failure in birds, creatinine level was not significantly ( $p > 0.05$ ) influenced by PTE, this shows that the integrity of the animal's kidney is maintained. According to [48], creatinine builds up in the blood can occur once the kidney is not filtering the blood effectively. However, all the values of the serum biochemical constituents studied fall within the ranges for broilers reported by [49] on the serum biochemical values of indigenous chickens in Al-Ahsa, Saudi Arabia.

SGPT and SGOT level decrease as the level of PTE increases in the treatment. According to [50], SGPT and SGOT respond the presence of toxic substance in the blood. PTE has revealed to be non-toxic to the animal, similar observation was made by when *Parkia biglobosa* leaf extract was feed to broiler chickens at different levels.

## Conclusion

Secondary plant metabolites or bioactive chemicals gives plants the strength to perform multiple biological activities such as antimicrobial, anti-inflammatory, antiviral, antioxidant and antifungal properties. PTE is a potential phytogetic feed additive because of their abundant phytochemicals and was found to be loaded with several minerals with allows birds to attain optimal performance. It can be included in the diet of broiler chicks up to 80 ml per litre with no detrimental effect on the performance and health status of birds.

## References

1. Lina Sernaite (2017) Plant extracts: antimicrobial and antifungal activity and appliance in plant protection (Review). Lithuanian Journal of Agriculture and Forestry 3: 58-66.
2. Cherkupally R, Kota SR, Amballa H, Reddy BN (2017) *In vitro* antifungal potential of plant extracts against *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Annals of Plant Sciences 6: 1676 – 1680.
3. Rates SMK (2001) Plant as source of drugs. Toxicon Journal, 39: 603-613.
4. WHO- World Health Organization (1992) Quality control methods for medicinal plant materials. Geneva.
5. Ighodaro I, Agunbiade SO, Omole JO, Kuti OA (2012) Evaluation of the chemical, nutritional, antimicrobial and antioxidant vitamin profiles of *Piliostigma thonningii* leaves. Research Journal of Medicinal Plant 6: 537-543.
6. Akladahunsi AA, Salawu SO (2005) Phytochemical screening and nutrient-anti-nutrient composition of selected tropical green leafy vegetables. African Journal of Biotechnology 4: 497-501.
7. Jimoh FO, Oladiji AT (2005) Preliminary studies on *Piliostigma thonningii* seeds: proximate analysis, mineral composition and phytochemical screening. African Journal of Biotechnology 4: 1439-1442.
8. Akinpelu DA, Obuotor EM (2000) Antibacterial activity of *Piliostigma thonningii* stem bark. Fitoterapia 71: 442-443.
9. Igoli JO, Ogaji OG, Tor-Anyin TA, Igoli NP (2005) Traditional medicine practice amongst the Igede people of Nigeria part II. African Journal of Traditional Complementary 2: 134-152.
10. Fakae BB, Cambell AM, Barrett J, Scott IM, Teesdale-Spittle PH, *et al.* (2000) Inhibition of glutathione transferase from parasitic nematodes by extracts from traditional medicinal plants. Phytother Res 14: 630-634.
11. Dabofunjo OP, Adebayo AH, Aliyu R, Garba IH (2012) The effects of methanolic extract of *Piliostigma thonningii* leaf on lipid profile of rats. International Journal of Pharmacology, 2: 501-508.
12. Dabofunjo OP, Adebayo AH, Aliyu R, Garba IH (2013) The effects of ethanolic extract of *Commiphora Africana* on lipid profile of rats. International Journal of Pharmacology, 2: 618-622.
13. Alagbe JO (2019) Growth performance and hemato-biochemical parameters of broiler chickens fed different levels of *Parkia biglobosa* leaf extracts. Academic Journal of Life Sciences 5: 107-115.
14. Jain NC (1986) Schalm's veterinary hematology. 4<sup>th</sup> ed. Philadelphia: Lea and Febiger.
15. Scott HM (1965) Measurement of the Amino Acid Content of Fishmeal protein by Chick Growth Assay. Journal of Poultry Science pp: 395-465.
16. AOAC (2000) Official methods of analysis. 25<sup>th</sup> edition, Association of Official Analytical Chemists. Washington D.C, USA.
17. Harbone JD (1973) Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall, London, 279.



18. Odebiyi A, Sofowora AE (1978) Phytochemical screening of Nigerian medicinal plant. Part III, *Lloydia* 41: 234-246.
19. Van-Burden TP, Robinson WC (1981) Formation of complexes between protein and tannic acid. *Journal of Agriculture and Food Chemistry* 1:77.
20. Boham BA, Kocipai AC (1974) Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium vaticulatum* and *V. calycinium*. *Pacific Journal of Science* 48: 458-463.
21. Duncan DB (1955) Multiple range and multiple F-test. *Biometrics* 11: 1-42.
22. Ayoola AA, Yusuf AO, Oki DG (2016) Phytochemical screening and proximate analysis of *Newbouldia laevis* and *Allium sativum*. *Nigerian J Anim Sci* 1: 242-256.
23. Norton BW (1994) The Nutritive value of tree legumes in: Forage tree legumes in Tropical Agriculture. Gutteride, R.C and Shelton H.M (Ed.) *Cab International* 177: 202-215.
24. Oluwaseun, Zack Agbendeh, Adikwu Jacob G (2013) Comparative studies of phytochemical screening of *Ficus sycomorus* linn stem bark extract and *Piliostigma thonningii* root extract. *Asian Journal of Plant Science and Research* 3: 69-73.
25. Alagbe JO (2019) Growth response and bacteria count of broiler starter given *Delonix regia* leaf extract as a natural alternative to antibiotics. *Sumerianz Journal of Agriculture and Veterinary* 2: 76-81.
26. Alagbe JO, Oluwafemi RA (2019) Performance and hematological parameters of broilers given different levels of dried *lemon grass* and garlic extract. *Research in: Agriculture and Veterinary Sciences* 3: 102-111.
27. Kumar V, Amit K (2010) Role of phytate and phytase. <http://www.scribd.com/dietary>.
28. Ojewuyi OB, Ajiboye TO, Adebajo EO, Balogun A, Mohammed AO (2014) Proximate composition, phytochemical and mineral contents of young and mature *Polyalthia longifolia* leaves. *Fountain Journal of Natural and Applied Sciences* 3: 10-19.
29. Saleem R, Ahmed M, Ahmed SI, Azeem M (2005) Hypotensive activity and toxicology of constituents from root bark of *Polyalthia longifolia*. *Phytother Res* 19: 881-884.
30. Adisa RM, Choudhary EA, Adenoye GA, Olorunsogo OO (2010) Hypoglycaemic and biochemical properties of *Cnestis ferruginea*. *Afr J Tradit Complement Altern Med* 7: 185-194.
31. Olorede BR, Longe OG (2000) Effect of replacing palm kernel cake with shear butter cake on quality characteristics, hematology and serum chemistry of laying hens. *Nigerian Journal of Animal Production* 27: 19-23.
32. Etim NN, Williams ME, Enyenihi GE, Udo MD, Offiong EE (2013) Hematological parameters: indicators of the physiological status of farm animals. *British Journal of Science* 10: 33-45.
33. Azeez OI, Oyagbemi AA, Oyewale JO (2009) Diurnal fluctuation in hematological parameters of the domestic fowl in the hot humid tropics. *International Journal of Poultry Science* 8: 247-251.
34. Elagib HAA, Ahmed ADA (2011) Comparative study on hematological values of blood of indigeneous chickens in Sudan. *Asian Journal of Poultry Science* 5: 41-45.
35. Obikaonu HO, Okoli IC, Opara MN, Okoro MO, Ogbuewu IP, *et al.* (2011). Hematological and serum biochemical indices of starter broilers fed *neem* (*Azadirachta indica*) leaf meal. *Online Journal of Animal and Feed Research* 1: 150-154.
36. Talebi AS, Asri-Rezaei S, Rozeh-Chai R, Sahraei R (2005) Comparatives studies on hematological values of broiler strains (Ross, Cobb, Arbo-acres, Arian). *International Journal of Poultry Science* 4: 573-579.
37. Abdi-Hachesoo B, Talebi A, Asri-Razaei S (2011) Comparative study on blood profiles indigenous and Ross-308 broiler breeders. *Global Vet* 7: 238-241.
38. Isaac LJ, Abah G, Akpan B, Ekaette IU (2013) Hematological properties of different breeds and sexes of rabbits (p.24-27). *Proceedings of the 18<sup>th</sup> Annual Conference of Animal Science Association of Nigeria*.
39. Ugwuene MC (2011) Effect of dietary palm kernel meal for *maize* on the hematological and serum chemistry of broiler turkey. *Nigerian Journal of Animal Science* 13: 93-103.
40. Chineke CA, Ologun AG, Ikeobi CON (2006) Hematological parameters in rabbit breeds and crosses in humid tropics. *Pakistan Journal of Biological Sciences* 9: 2102-2106.
41. Soetan KO, Akinrinde AS, Ajibade TO (2013) Preliminary studies on the hematological parameters of cockerels fed raw and processed guinea corn (*Sorghum bicolor*) Pg. 49-52. *Proceedings of 38<sup>th</sup> Annual Conference of Nigerian Society of Animal Production*.
42. Gotoh S, Takennako O, Vatanabe K, Kawamoto R, Watanabe T (2001) Hematological values and parasitic fauna in free ranging *Macaca hecki* and the *Macaca tonkeanai hecki* hybrid group of Sulawesi Island. *Indonesia Primates* 6: 91.
43. Butterworth AE (1999) Cell mediated damage in helminthes. *Adv Parasitol* 23: 143-235.
44. Oleforuh-Okoleh UV, Ogunnupebi TJ, Iroka CJ (2015) *Asian Journal of Poultry Science* 9: 242-249.
45. Akintomide AA, Joseph OG, Onibi GE (2018) Hematology and serum biochemistry of cockerels fed diets containing *neem* leaf meal. *Applied Tropical Agriculture*. 23: 12-16.
46. Dey B, Chowdhury SD, Bulbul SM, Chowdhury BLD (2011) Efficacy of *neem* leaf meal as a hypocholesterolemic dietary additive in laying pullets. *Bangladesh Journal of Animal Science*. 40: 13-17.
47. Borges LP, Borges VC, Moro AV, Nogueira CW, Rocha JBT, *et al.* (2005) Protective effect of diphenyl diselenide on acute liver damage induced by 2-nitropropane in rats. *Toxicology* 210: 1-8.
48. Ibrahim Albokhadaim (2012) Hematological and some biochemical values of indigenous chickens in Al-Ahsa, Saudi Arabia during summer season. *Asian Journal of Poultry Science* 6: 138-145.
49. Alagbe JO (2017) Effect of miadasan as a dietary supplement on performance, carcass characteristics and blood profile of broiler chickens. *Scholarly Journal of Agricultural Science* 7: 27-33.
50. Oloyede OB, Minari JB, Muhammad NO (2010) Evaluation of growth characteristics and hematological indices of broiler chicks fed raw and processed *Bambara groundnut seed* as a component of poultry feed. *International Journal of Poultry Science* 9: 625-655.



- Advances In Industrial Biotechnology | ISSN: 2639-5665
- Advances In Microbiology Research | ISSN: 2689-694X
- Archives Of Surgery And Surgical Education | ISSN: 2689-3126
- Archives Of Urology
- Archives Of Zoological Studies | ISSN: 2640-7779
- Current Trends Medical And Biological Engineering
- International Journal Of Case Reports And Therapeutic Studies | ISSN: 2689-310X
- Journal Of Addiction & Addictive Disorders | ISSN: 2578-7276
- Journal Of Agronomy & Agricultural Science | ISSN: 2689-8292
- Journal Of AIDS Clinical Research & STDs | ISSN: 2572-7370
- Journal Of Alcoholism Drug Abuse & Substance Dependence | ISSN: 2572-9594
- Journal Of Allergy Disorders & Therapy | ISSN: 2470-749X
- Journal Of Alternative Complementary & Integrative Medicine | ISSN: 2470-7562
- Journal Of Alzheimers & Neurodegenerative Diseases | ISSN: 2572-9608
- Journal Of Anesthesia & Clinical Care | ISSN: 2378-8879
- Journal Of Angiology & Vascular Surgery | ISSN: 2572-7397
- Journal Of Animal Research & Veterinary Science | ISSN: 2639-3751
- Journal Of Aquaculture & Fisheries | ISSN: 2576-5523
- Journal Of Atmospheric & Earth Sciences | ISSN: 2689-8780
- Journal Of Biotech Research & Biochemistry
- Journal Of Brain & Neuroscience Research
- Journal Of Cancer Biology & Treatment | ISSN: 2470-7546
- Journal Of Cardiology Study & Research | ISSN: 2640-768X
- Journal Of Cell Biology & Cell Metabolism | ISSN: 2381-1943
- Journal Of Clinical Dermatology & Therapy | ISSN: 2378-8771
- Journal Of Clinical Immunology & Immunotherapy | ISSN: 2378-8844
- Journal Of Clinical Studies & Medical Case Reports | ISSN: 2378-8801
- Journal Of Community Medicine & Public Health Care | ISSN: 2381-1978
- Journal Of Cytology & Tissue Biology | ISSN: 2378-9107
- Journal Of Dairy Research & Technology | ISSN: 2688-9315
- Journal Of Dentistry Oral Health & Cosmesis | ISSN: 2473-6783
- Journal Of Diabetes & Metabolic Disorders | ISSN: 2381-201X
- Journal Of Emergency Medicine Trauma & Surgical Care | ISSN: 2378-8798
- Journal Of Environmental Science Current Research | ISSN: 2643-5020
- Journal Of Food Science & Nutrition | ISSN: 2470-1076
- Journal Of Forensic Legal & Investigative Sciences | ISSN: 2473-733X
- Journal Of Gastroenterology & Hepatology Research | ISSN: 2574-2566
- Journal Of Genetics & Genomic Sciences | ISSN: 2574-2485
- Journal Of Gerontology & Geriatric Medicine | ISSN: 2381-8662
- Journal Of Hematology Blood Transfusion & Disorders | ISSN: 2572-2999
- Journal Of Hospice & Palliative Medical Care
- Journal Of Human Endocrinology | ISSN: 2572-9640
- Journal Of Infectious & Non Infectious Diseases | ISSN: 2381-8654
- Journal Of Internal Medicine & Primary Healthcare | ISSN: 2574-2493
- Journal Of Light & Laser Current Trends
- Journal Of Medicine Study & Research | ISSN: 2639-5657
- Journal Of Modern Chemical Sciences
- Journal Of Nanotechnology Nanomedicine & Nanobiotechnology | ISSN: 2381-2044
- Journal Of Neonatology & Clinical Pediatrics | ISSN: 2378-878X
- Journal Of Nephrology & Renal Therapy | ISSN: 2473-7313
- Journal Of Non Invasive Vascular Investigation | ISSN: 2572-7400
- Journal Of Nuclear Medicine Radiology & Radiation Therapy | ISSN: 2572-7419
- Journal Of Obesity & Weight Loss | ISSN: 2473-7372
- Journal Of Ophthalmology & Clinical Research | ISSN: 2378-8887
- Journal Of Orthopedic Research & Physiotherapy | ISSN: 2381-2052
- Journal Of Otolaryngology Head & Neck Surgery | ISSN: 2573-010X
- Journal Of Pathology Clinical & Medical Research
- Journal Of Pharmacology Pharmaceutics & Pharmacovigilance | ISSN: 2639-5649
- Journal Of Physical Medicine Rehabilitation & Disabilities | ISSN: 2381-8670
- Journal Of Plant Science Current Research | ISSN: 2639-3743
- Journal Of Practical & Professional Nursing | ISSN: 2639-5681
- Journal Of Protein Research & Bioinformatics
- Journal Of Psychiatry Depression & Anxiety | ISSN: 2573-0150
- Journal Of Pulmonary Medicine & Respiratory Research | ISSN: 2573-0177
- Journal Of Reproductive Medicine Gynaecology & Obstetrics | ISSN: 2574-2574
- Journal Of Stem Cells Research Development & Therapy | ISSN: 2381-2060
- Journal Of Surgery Current Trends & Innovations | ISSN: 2578-7284
- Journal Of Toxicology Current Research | ISSN: 2639-3735
- Journal Of Translational Science And Research
- Journal Of Vaccines Research & Vaccination | ISSN: 2573-0193
- Journal Of Virology & Antivirals
- Sports Medicine And Injury Care Journal | ISSN: 2689-8829
- Trends In Anatomy & Physiology | ISSN: 2640-7752

Submit Your Manuscript: <https://www.heraldopenaccess.us/submit-manuscript>