Nig. J. Anim. Prod. 2019, 46(2):164 - 175
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Haematology, serum biochemistry and organ histopathology of broiler chickens fed graded dietary levels of *Gongronema latifolia* (Utasi)

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Abstract

An experiment was carried out to evaluate effects of different dietary levels of Gongronema latifolia leaf meal (GLLM) on blood parameters and histopathology of broiler chickens. Five experimental diets were formulated. Diet 1 (control) contained 0% GLLM while diets 2, 3, 4 and 5 contained 2.5%, 5.0% 7.5 and 10.0% GLLM respectively. Each diet was fed to a group of 30 broilers (one week old) for 49 days. A dietary inclusion level of 10% GLLM significantly (P<0.05) reduced RBC concentration but had no significant (P>0.05) effect on other haematological indices. There was a significant (P<0.05) reduction of serum glucose at7.5% and10% and of AST at5%,7.5% and10%. Bilirubin increased significantly (P<0.05) 5%, 7.5, 10% and 2.5% dietary levels of GLLM. Other serum parameters were not affected (P>0.05) by the treatments. There were no indications of pathologic significance in the liver, kidney, proventriculus or pancreas. An inclusion level of 10% Gongronema latifolia leaf meal in diet can therefore support normal broiler production.

Keywords: *Congronema_latifolia,* broiler chickens, leaf meal, blood parameters, histopathology

Introduction

Broiler chickens (*Gallus domesticus*) are domesticated fowl, bred and raised specifically for meat. Haruna and Hamidu (2004) noted that they are characterised by fast growth rate, high feed conversion ratio, high turnover and quick return on investment. The high cost of mono-gastric livestock production in most developing countries is as a result of exorbitant cost of feed ingredients like cereal grains and protein concentrates (Esonu_*et al*., 2003; Obih and Ekenyem, 2010; Madubuike and Ekenyem, 2001). This has resulted in many feed producers being forced out of production, or in the alternative producing low quality feeds (Nsa *et al*., 2007). Currently the focus in the monogastric livestock feeding is on the use of nonconventional feedstuffs. These include those which can either substitute directly for cereal grains and/or protein concentrates, or can be included at a certain level to attain a comparable quality of production with the conventional ones, but must not be deleterious to animal health (Obih, 2009).

Reports from (Tewe, 2003) and Adegbola (2004) among others have shown that various alternative feedstuffs have been fed to poultry with remarkable results. Some of these alternative sources include the leaf meals of some tropical legumes and browse plants, rich in nutrients like vitamins, minerals and oxycarotenoids (Vohra *et al*., 1972; Okoli_*et al*., 2001 and 2003; Esonu_*et al*., 2002, 2004 and 2005).

Hematological and serum biochemical components of the blood of an animal have been found to be influenced by the quantity and quality of its feed (Akumitimi, 2004). Therefore, blood analysis acts as a reflector of the health status of exposed animals to toxicants and other conditions (Olafedehan *et al., 2010*). As a matter of fact, studies on the nutrition of animals need to go beyond their growth response, nitrogen balance, and efficiency of feed utilization. It is also important to monitor the influence of nutrition on the metabolism of the cells in

the various tissues. It is well established that haematological parameters are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as health status of farm animals (Oyawoye and Ogungunle, 1998; Isaac *et al., 2013;* Ikeobi *et al., 2011*). On the other hand, serum biochemical analysis has been reported by many researchers to provide information on the integrity of internal organs (liver and kidney), electrolytes, proteins and nutritional or metabolic parameters (Iyayi and Tewe, 1998; Awosanya_*et al.,* 1999; Iyayi, 2001; Yalcin *et al*., 2012). Similarly, histopathology constitute histological procedures aimed to provide good quality sections that can be used for a light microscopic evaluation of animal (or human) tissue changes in either spontaneous or induced diseases. A combination of blood analysis of the experimental birds and the histopathological evaluation of the tissues of their internal organs can provide some information on the health status of the birds after the feeding trial with *G. latifolia*.

Considering the combinations of ingredients used by the traditional animal health practitioners, it is likely that additive, synergistic, and nutritional effects of leaf meals might be involved in alleviating livestock maladies. Many phytochemicals have been shown to be bio-active, and have been useful as chemotherapeutic agents, pesticides, food/feed additives and other biologicals (Osuala_*et al., 2005;* Onwukwe, 2000; Ukorebi, 2011). *Gongronema latifolia (utazi)* is a wild tropical creeping plant with lush deep green vegetation. The vegetation is perennial when it grows in a swampy area or inland valley, otherwise, it is deciduous. The plant plays a significant role in ethnomedicine. Extracts from the leaf is used in treating malaria, stomach-ache and diarrhoea (Dalziel, 1956). Osuala_*et al* (2005) reported that methanolic extract of *C. latifolia* exhibited marked activity against *Pseudomonas aeruginosa and E.coli* with both having a minimum inhibitory concentration (MIC) of 15.625 mg/ml in an *in vitro* study. Their investigations have new evidence to corroborate an earlier work by Dalxiel (1956), which reported the use of *C. latifolia* in the treatment of diarrhoea. *Gongronema latifolia* is abundant in Cross River State, Nigeria. It can readily be found in its southern part, up to areas beyond the central region of the state. This location lies along longitudes 8° and 9° East, and latitudes 6° and 7° North of the equator, with an ambient temperature range of 21°C – 30°C and annual rainfall of 500 – 1070mm (Mfam, 2002). The forest areas of Akamkpa, Biase, Ugep, Mkpani, and Obubra among others are rich reservoirs of the plant.

Materials and methods

The research was carried out at the poultry unit of the Teaching and Research Farm of the School of_Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri.

Preparation of Gongronema_latifolia_Leaf meal (GLLM)

Fresh leaves of *G. latifolia* were harvested from the forest area of Ovonum in Obubra Local

Government Area of Cross River State, Nigeria. Harvested leaves were air-dried under a shade to prevent_inactivation of its chemical constituents by direct insolation. The drying process was done between December and January for 5 - 7 days until the leaves became crispy to_touch. The dried leaves were milled to particle sizes that would pass through a 2mm sieve, in a hammer mill, and thereafter stored in air-tight plastic containers under cool dry conditions prior to use.

Experimental diets

The experiment was in two phases, the starter and finisher phases respectively. Five broiler starter experimental diets were formulated such that inclusion of GLLM was at 0.0, 2.5, 5.0, 7.5 and 10% levels, respectively. White maize was used as the major energy source for the rations. At the second phase of the trial (finisher phase), the experimental diets were adjusted to broiler finisher diets by increasing the energy level and reducing protein content. For a preliminary investigation such as this, 10% inclusion level of the leaf meal was considered moderate. The ingredients composition of the experimental diets is shown in Tables 1 and 2.

Experimental birds and design

A total of one hundred and fifty day-old Anak broiler chicks used in the trial was acquired from Chike and Sons ventures, Owerri, Imo State. They were raised on commercial broiler starter diet for one week, after which they were randomly distributed into five groups of thirty birds each, and assigned to the five experimental diets in completely randomized design (CRD). Each group was sub-divided into three replicates of ten birds each and housed in separate pens. Normal brooding was carried out for three weeks. Feed and water were provided *ad libitum* for all experimental birds all through the experimental period. This was accompanied by necessary prophylactic medications and vaccinations. The starter phase of the 28 days, followed by a finisher phase for another 28 days.

Table 1: Ingredient composition of the experimental diets (Broiler Starter)

	Dietary Levels of GLLM (%)				
Ingredients	**0.0**	**2.5**	**5.0**	**7.5**	**10.0**
Maize (White)	50.00	48.00	46.00	44.00	42.00
Soybean Meal	28.00	28.00	28.00	28.00	28.00
GLLM*	0.00	2.50	5.00	7.50	10.00
Wheat Offals	8.00	7.75	7.75	6.75	6.25
Palm Kernel Cake	5.00	5.00	5.00	5.00	5.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Blood Meal	3.00	3.00	3.00	3.00	3.00
Bone Meal	3.00	3.00	3.00	3.00	3.00
Common Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Vitamin / Trace	0.25	0.25	0.25	0.25	0.25
Mineral premix**					
Total	**100**	**100**	**100**	**100**	**100**
Calculated Chemical Composition					
Crude protein	22.72	22.79	22.86	22.93	23.0
Crude fibre	4.15	4.11	4.11	4.12	4.13
Ether extract	3.68	3.65	3.63	3.60	3.57
Ash	3.46	3.56	3.66	3.75	3.85
Calcium	1.33	1.32	1.32	1.32	1.32
Phosphorus (available P)	0.76	0.77	0.78	0.78	0.79
L-methionine	0.62	0.63	0.65	0.66	0.69
L-lysine	1.60	1.67	1.79	1.88	1.97
ME (Kcal/Kg)	2873.45	2868.01	2856.56	2857.12	2851.67

** To provide the following per kilogram of feed: Vit. A, 10,000iu; Vit. D_3, 2000iu; Vit. E, 5iu; Vit.K, 2mg; Riboflavin, 4.20mg; Vit. B_{12}, 0.01mg; Panthotenic acid, 5mg; Nictotnicacid, 20mg; Folic acid, 0.5mg; choline, 3mg; Mg, 56mg; Fe, 20mg; Cu, 10mg; Zn, 50mg; Co,125mg.
*GLLM: *Gongronema latifolia Leaf Meal.*

Table 2: Ingredient composition of the experimental broiler finisher diets

	Dietary Levels of GLLM (%)				
Ingredients	**0.0**	**2.5**	**5.0**	**7.5**	**10.0**
Maize (White)	60.00	58.00	56.00	54.00	52.00
Soybean Meal	16.00	16.00	16.00	16.00	16.00
GLLM*	-	2.50	5.00	7.50	10.00
Wheat Offals	8.00	7.50	7.70	6.50	6.00
Palm Kernel Cake	7.00	7.00	7.00	7.00	7.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Blood Meal	3.00	3.00	3.00	3.00	3.00
Bone Meal	3.00	3.00	3.00	3.00	3.00
Common Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Vitamin / Trace	0.25	0.25	0.25	0.25	0.25
Mineral premix**					
Total	**100**	**100**	**100**	**100**	**100**
Calculated Chemical Composition					
Crude protein	19.04	19.11	9.18	19.25	19.33
Crude fibre	3.81	3.80	3.79	3.78	3.76
Ether extract	3.78	3.75	3.73	3.70	3.67
Ash	2.96	3.06	3.16	3.26	3.26
Calcium	1.30	1.30	1.30	1.30	1.30
Phosphorus (available P)	0.70	0.71	0.72	0.72	0.73
L-methionine	0.57	0.59	0.60	0.62	0.64
L-lysine	1.31	1.40	1.49	1.58	1.67
ME (Kcal/Kg)	2936.35	2930.91	2925.46	2920.02	2914.56

** To provide the following per kilogram of feed: Vit . A, 10,000iu; Vit. D_3, 2000iu; Vit. E, 5iu; Vit.K, 2mg; Riboflavin, 4.20mg; Vit. B_{12}, 0.01mg; Panthotenic acid, 5mg; Nictotnic acid, 20mg; Folic acid, 0.5mg; choline, 3mg; Mg, 56mg; Fe, 20mg; Cu, 10mg; Zn, 50mg, CO_2, 125mg.
GLLM: Gongronema latifolia Leaf Meal.*

Data collection

At the end of the feeding trial period of 56 days, three birds per treatment were randomly selected for bleeding. Blood was sampled from the punctured webal sub-clavian vein with a 5ml scalp vein needle. A blood volume of 7ml was aspirated from each bird, 2ml of which was discarded into ethylene di-amine tetra acetic acid (EDTA) treated Bijou bottles for haematological assay, while the remaining 5ml was allowed to coagulate in plain vial bottles (without anti-coagulant), to produce serum for blood chemistry evaluations.

Haematology

Un-coagulated blood samples were analysed within three hours (3hours) of their collection for total erythrocyte (RBC) and leukocyte (WBC) counts, hematocrit (PCV), hemoglobin concentration (HC) and erythrocyte sedimentation rate (ESR). Other haematological indices (mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC)) were calculated from results obtained. Erythrocyte sedimentation rate (ESR) was determined within six hours of sample collection.

The concentration of blood haemoglobin in the test samples were estimated according to the cyanomethaemoglobin method of Alexander and Griffiths (1993). All haematological analysis were carried out in

accordance with standard methods described by Ukorebi (2011)

Serum biochemistry

The samples separated for serum biochemistry were centrifuged at 3000 rpm for ten minutes to separate serum. Thereafter, the harvested sera were used for evaluation of total serum protein (TSP) and serum albumin (SA). Globulin was determined by calculation.

Cholesterol and other biochemical parameters such as creatinine and urea concentration were also determined accordingly.

The standard flame photometry using Gallenkamp analysis was used to determine serum sodium ion (Na^{+}) and potassium ion (K^{+}), while Calcium ion (Ca^{2+}) was determined by atomic absorption spectrophotometry; serum phosphate ion (HPO_4^{2-}) was measured using trichlotoacetic acid, ammonium molybdate and metol to develop blue colour and read thereafter in a Buck 205 spectrophotometer. Other serum parameters monitored included aspartate aminotransferase (AST), Alanine aminotransferse (ALT) and Alkaline phosphatase (ALP). The activities of these enzymes were determined using spectrophotometric methods. Total or unconjugated bilirubin (TB) and direct or conjugated bilirubin (DB) were alsodetermined. All serum biochemical analyses were done according to the methods described by Ukorebi (2011).

Histopathological study of experimental birds

This study was executed at the Department of Anatomy Laboratory, College of Medical Sciences, University of Calabar. At the end of the feeding trial, four birds were randomly selected per treatment for histopathological studies. These were sacrificed by cervical bone dislocation, after which the liver, pancreas, proventriculus and kidney were harvested. These were cut and fixed in bouin's fixative for 24 hours, then hydrated, cleared and infiltrated in molten paraffin. Thereafter, the tissues were embedded in pure paraffin wax and sectioned at 5 - 6 micron in a microtom. They were then stained with haematoxilin and eosin and subsequently examined by light microscopy for histopatological changes.

Statistical analyses

Data on haematology and serum biochemistry were subjected to one-way analysis of variance (ANOVA) as outlined by Snedecor and Cochran (1978). Where ANOVA detected significant treatment effects, means were separated using the Duncan's New Multiple Range Test (DNMRT) as outlined by Little and Hills (1978).

Results

Haematological indices

The effects of the different levels of *Gongronema latifolia* leaf meal on the haematological parameters of experimental birds are summarized in Table 4. The results showed Hb, ESR, PCV), MCV, MCH and MCHC were not significantly ($P > 0.05$) affected by the treatments. However, WBC and RBC values differed significantly ($P < 0.05$) among the treatment groups.

Serum biochemical indices

The effects of different dietary levels of *Gongronema latifolia* leaf meal on the serum biochemical parameters of experimental birds are summarized in Table 5. There were significant ($P<0.05$) treatment effects for glucose, AST., total bilirubin and direct bilirubin. All other serum parameters measured were statistically ($P > 0.05$) similar.

Histopathology

Effects of GLLM on the histopathological structure of the livers, kidneys proventriculus and pancreas of the experimental birds are presented in Table 6

The liver lobules across the treatments showed a central vein from where the

hepatocytes radiated out in hexagonal pattern. The hepatocytes were well stained. Some hepatocytes had more than one nucleus; the nuclei had the normal open faced conformation. The rounded glomerulus, characterised by a tuft of capillaries was visible in the renal cortex of the kidney. Its endothelial cells were outlined. There was a narrow clearance between the renal (glomerular) capsule and the glomerulus, thus the urinary space was outlined. The proximal and distal convoluted tubules were differentiated and their nuclei were prominent. Tissues of T_i birds showed normal proventricular mucosal tissues. However, the sub-mucosa and muscularis externa indicated degenerative changes. Muscle tissues were atrophic. In T_5 birds proventricular tissues showed mildly distorted cyto-architecture in the mucosal layer. However, the muscularis were intact. Other treatments indicated no marked degenerative changes in their tissue sections. Pancreas tissues of T_1 birds showed diffused acinar tissue necrosis with islet cells degeneration. No inflammatory cells were seen. Tissue sections in T_5 also showed similar degenerative changes.

Table 3: Chemical composition of GLLM

Nutrients (%)	Concentration
Moisture	8.04
Dry matter Dm	91.96
Crude protein (% of Dm)	91.96
Ether extract (% of Dm)	2.84
Ash (% of Dm)	6.26
Crude fibre extractives (% of Dm)	60.39
Metabolizable energy (Kcal/Kg)	2903.41
Calcium (Mg/100g)	10.8
Magnesium (Mg/100g)	45.0
Potassium (Mg/100g)	486.0
Sodium (Mg/100g)	3.86
Phosphorus (Mg/100g)	395.3

Table 4: Effects of different dietary levels of GLLM on haematological indices of broiler chickens

	Dietary Levels of GLLM (%)					
Parameters	**T_1 (0%)**	**T_2 (2.5%)**	**T_3 (5.0%)**	**T_4 (7.5%)**	**T_5 (10%)**	**SEM**
Hb (g/dl)	9.27	9.87	9.77	8.97	8.90	0.52
RBC (10^9mm)	2.28^{ab}	2.35^{a}	2.44^{a}	2.43^{a}	2.27^{ab}	0.04
WBC (mm^3)	41984.33^{ab}	52394.67^{a}	39296.0^{b}	36430.67^{b}	26602.67^{c}	7969.55
ESR (mm/hr)	2.00	3.00	2.33	3.67	4.67	0.68
PCV (%)	26.67	27.67	29.00	27.67	27.00	1.57
MCV (FI)	116.83	117.77	118.70	122.37	111.07	6.39
MCH (g/dl)	40.61	37.75	39.96	39.65	36.61	2.02
MCHC (d/dl)	34.67	32.05	33.62	32.47	35.34	0.96

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

Table 5: Effects of different dietary levels of GLLM on the serum biochemical indices of broiler chickens

Parameters	Dietary Levels of GLLM (%)					SEM
	T_1 (0%)	T_2 (2.5%)	T_3 (5.0%)	T_4 (7.5%)	T_5 (10%)	
Total protein (g/dL)	2.99	3.31	3.21	3.13	2.99	0.16
Albumin (g/dL)	1.79	1.89	2.02	1.96	1.57	0.17
Globulin (mg/dL)	1.27	1.42	1.20	1.25	1.33	0.22
Cholesterol (mg/dL)	74.86	77.30	80.40	76.30	77.52	5.43
Creatinine (mg/dL)	0.14	0.18	0.31	0.21	0.21	0.05
Urea (mg/dL)	15.57	18.48	24.62	22.04	21.76	1.97
Glucose (mg/dL)	118.33^{a}	104.79^{a}	105.49^{a}	88.63^{b}	83.61^{b}	6.35
Sodium (Na) (mmol/l)	127.0	133.87	138.70	136.87	137.20	2.69
Calcium (Ca) (mg/dL)	9.81	10.86	10.05	9.19	10.79	0.64
Potassium (K) (mmol/Lt))	3.03	3.77	3.53	3.43	3.33	0.56
AST (U/L)	76.0^{a}	65.33^{a}	65.0^{a}	51.33^{ab}	40.33^{ab}	7.40
ALT (U/L)	4.33	5.67	4.67	5.0	6.67	1.27
Total Bilirubin (mg/dL)	0.09^{b}	0.21^{ab}	0.37^{a}	0.32^{a}	0.31^{a}	0.06
Direct Bilirubin (mg/dL)	0.03^{b}	0.06^{ab}	0.17^{a}	0.15^{a}	0.15^{a}	0.04

^{ab}Means in the same row with different superscripts are significantly different (P<0.05).

Table 6 : Effect o f different dietary levels of GLLM on histopathological structure of kidneys, livers, pancreas and proventriculus of the experimental broilers chickens

Parameters	Dietary Levels of GLLM (%)				
	0.0	2.5	5.0	7.5	10.0
Diffused acinar tissue necrosis (Pancreas)	+++	-	-	-	++
Islet cells degeneration (Pancreas)	++	-	+	-	++
Muscularisexterna degeneration (Proventricular)	+	-	-	-	+
Nephritis (kidney)	-	-	-	-	-
Intercellular reaction (kidney)	++	+	-	-	-
Hepatic necrosis (liver)	+	-	+	-	-
Mononuclear reaction(liver)	+	-	-	-	-
Fatty liver change	+	-	-	-	-

\+ Relative presence of histopathological structure
\- Absence of histopathalogical structure.

Discussion

Haematological indices

There were no significant differences (P>0.05) in Hb values recorded among the treatment groups. The values were within literature range as reported by Mitruka and Ransley (1977). Erythrocyte (RBC) values were significantly (P<0.05) affected by the treatments. T_3 (5.0%) T_4(7.5%) and T_2(2.5%) GLLM, respectively, recorded the highest values which were statistically similar. These were followed by T_1(control) and T_5(10.0% GLLM) which were within the range of standard values reported by Mitruka and Ransley (1977). The higher RBC values recorded by T_2 to T_4 are suggestive of the blood building potential of GLLM up to 10% inclusion level for broiler birds. This agrees with the reports of Ezekwesili (2005). Packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell

haemoglobin concentration) were statistically similar (P>0.05) across the treatments. There were also no significant differences (P>0.05) among the treatment groups in erythrocyte sedimentation rate (ESR). White blood cell (WBC) value was highest at 2.5% dietary level, followed by the control, T_3, and T_4, respectively. The values dropped significantly (P<0.05) at T_5(10%) dietary level. Proliferation of leucocytes in the system is body defence reaction against invading pathogenic microbes. Since the level of production of the anti-bodies is commensurate to the magnitude of infection challenge, this result suggests that *G. latifolia* has anti-bacterial properties. This result corroborates the findings of Dalziel (1956) and Osuala *et al* (2005). All haematological parameters studied showed that the test diets were nutritionally adequate and safe when compared to the control diets.

Serum biochemical parameters

There were no significant differences (P>0.05) among the treatment groups in total serum protein. The similarity of serum protein values in the test diets to that of the control may be an index of similarity in their qualities. There were no significant differences (P>0.05) in the levels of serum albumin, serum globulin as well as serum creatinine across the treatments. Ezekwesili (2005) reported that severe malnutrition decreased albumin fraction in the blood. Globulin combines with albumin in the blood to promote normal water retention in the blood. Iyayi and Tewe (1998) observed that globulin and albumin as well as urea concentrations in the blood are indicators of the quality and quantity of proteins supplied in the diets. The values of the parameters obtained in this study and the normal health status of the birds across the treatments indicate that GLLM diets were safe and nutritionally adequate. The serum urea levels of the treatments were not statistically different (P>0.05). This indicates that their concentrations were normal. Normal urea level in the blood portrays adequate dietary protein quantity and quality. Elevated urea concentration in the blood, statistically above the normal range, is an indication of excessive deamination of serum protein as a result of its poor quality in terms of its amino acid profile and consequent inadequacy for normal protein metabolism in the animal system. Glucose levels obtained showed significant (P<0.05) treatment effects. T_1(control), T_2 (2.5% GLLM) and T_3(5.0%) GLLM recorded statistically higher glucose level than T_4 (7.5% GLLM) and T_5 (10.0% GLLM). The cause of the significantly lower glucose concentration in T_4 and T_5 is striking. This is probably because a significant proportion of the energy content of GLLM is contributed by its lipid portion. There were no significant differences (P>0.05) among the treatments in cholesterol levels. Cholesterol test is relevant in the evaluation of the risk of heart disease. The similarity of the values obtained in the study is suggestive of normal and safe concentration. Serum electrolytes (sodium, calcium and potassium) showed no significant differences (P>0.05) among the treatment groups. This is an indication of proper osmotic and electrolyte balance in the body fluid of the birds (Machebe *et al*., 2009) and the normal development of their skeletal system. Aspartate transaminase (AST) dropped significantly (P<0.05) at 7.5% and 10% CLLM dietary levels. There were no significant (P>0.05) differences among the treatment groups in alanine transaminase (ALT) and alkaline phosphatase (ALP). The liver is a prime site for the destruction of toxic compounds found in the blood. However, when the challenge of such deleterious factors become excessive, the cells of the organ are damaged and the above listed enzymes which are contained in it leak into the general circulation. The

serum concentration of the enzymes is therefore a function of hepatocellular intergrity and function. The similarity of the values for the serum enzymes from the experiment suggests that whatever toxic factor(s) are contained in GLLM were of mild concentrations and were adequately tolerated by the birds up to 10% dietary inclusion level. This observation agrees with the reports of several authors (Enemor *et al*., 2005; Ologhobo *et al*., 1993; Onwukwe, 2000; Owen *et al*., 2009) on serum or liver enzyme activities.

The values of the total (unconjugated) bilirubin and direct (conjugated) bilirubin were significantly ($P<0.05$) different among the treatments. Total bilirubin was highest at 5%, 7.5% and 10% dietary levels of GLLM. This was followed by 2.5% level while the control had the lower value. A similar trend was recorded for direct bilirubin. Bilirubin is primarily a product of the breakdown of the haem moiety of haemoglobin. The result of this study suggests that conditions which cause increased formation of bilirubin, such as destruction of red blood cells or decrease of its removal from the blood stream, such as liver disease occurred during the experiment. But since findings on the histopathological analysis of the livers of the experimental birds did not indicate damaged or diseased livers, the elevated bilirubin levels would be traceable to conditions which cause the destruction of the haem moiety of haemoglobin. The reason for the highest numerical value recorded at 5% rather than 10% dietary level is unclear.

Histopathological observations

No gross lesions of pathologic significance were observed in all the liver and kidney tissues studied. The normal structures and contours of the organs were maintained. The organs showed no discolouration, neither were there adhesions between them. The liver and kidney are the primary organs of biotransformation in animals. Clear lesion on these organs would be an obvious indication of the toxicity of the test material. The absence of the notable degenerative changes in these organs (for birds tested) is attributable to their normal roles in the elimination of metabolic wastes and toxins from the animal body.

However, there were some changes in focal mononuclear reactions of hepatocytes which were rather pronounced in control birds that had no test material in their diet. Also, necrotic spots observed (in hepatocytes), had no trend. Furthermore, intercellular reaction noticed in the kidney was more pronounced in the control birds. These changes therefore might have been caused by factors other than the test material.

Degenerative changes observed in the proventricular and pancreas tissues were common to both the control and T_5 birds. They were, however, not of pathologic significance.

Conclusion

The experiment suggests that dietary inclusion of GLLM up to 10% does not elicit deleterious effects on haematological indices of broiler chickens, but rather enhanced their blood building capacity. The serum and histopathological parameters studied indicate that the birds were not adversely affected by the dietary levels of GLLM used in the research.

It therefore follows that 10% dietary level of GLLM would support normal health and performance of broiler chickens.

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Received: 30th September, 2018
Accepted: 24th February, 2019