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Research Article
Effects of African Walnut (*Tetracarpidium conophorum*) Leaf and its Extract on Blood Profiles of Broiler Chickens

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Abstract

Background and Objective: The growing concerns of consumers on the use of antibiotic as a growth promoter in livestock feed have fueled the interest in alternative products, however, blood indices are viable indicator of the health status of birds given such alternative, Hence, this study was carried out to evaluate the effect of African Walnut (*Tetracarpidium conophorum*) leaf and its extract on haematology, serum chemistry and serum antioxidant enzymes of broiler chickens. **Materials and Methods:** A total of 135 unsexed 7 day old broiler chickens were used for 42 days trial. The birds were randomly distributed into 5 dietary treatments, each treatment included 3 replicates of 9 birds each. Five experimental diets were formulated with the inclusion of *Tetracarpidium conophorum* leaf powder (TCLP) and its extracts (TCLE) which includes: Control diet (T1), T1+250 mg TCLP kg/diet (T2), T1+500 mg TCLP kg/diet (T3), T1+25 mg TCLE kg/diet (T4) and T1+50 mg TCLE kg/diet (T5). Data were collected on haematology, serum biochemistry and serum antioxidant enzymes and subjected to one way of analysis of variance in a completely randomized design. **Results:** Birds fed diet T2 had the highest ($p<0.05$) high density lipoprotein (HDL) (50.77 mg dL^{-1}). Addition of 25 mg of TCLE significantly ($p<0.05$) increased the serum SOD (25.66 U mL^{-1}). Malondialdehyde was highest in the control birds (66.80 nmol mL^{-1}). **Conclusion:** It is therefore concluded that broiler chickens fed diet containing 250 mg TCLP had increased HDL cholesterol while all levels of addition of TCLP and TCLE had positive effects on the serum antioxidant enzyme activities.

Key words: African walnut leaf, broiler diet, haematology, herbal extract, poultry nutrition, serum antioxidant enzymes, serum biochemistry

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the past, growth-promoting antibiotics were used as feed additives and have played a key role in animal and poultry production. However, most of these antibiotics have been banned in many countries, particularly the European Union, because of public health concern regarding their residues in the animal products and the development of antibiotic resistance in bacteria[1]. Animal scientists and veterinarians are now searching for alternative sources of natural ingredients such as herbs or phytogenic plants to replace antibiotics. There were reports on the beneficial effects of herbs which are used as feed supplements or medicines in chickens[2]. Certain bioactive chemicals in phytogenic plants or herbs were reported to be responsible for their therapeutic (medicinal) benefits[2].

Many herbs have been used in the treatment of diseases and for revitalising body system in almost all ancient civilizations, the Egyptian, the Chinese and even Greek and Roman civilizations[3]. Kar *et al.*[4] have reported that several plant products are claimed and proved to possess analgesic and antipyretic properties. Majority of herbal plants are safe and economical. Generally, plant extracts have no problem of drug resistance. Herbs and medicinal plants also present a potential alternative to antibiotic growth promoters (AGP). Many plants have beneficial multifunctional aspects which are derived from their specific bioactive components. Herbs have been used for centuries to treat illness and improve health and still account for 80% of medical treatments in the developing world[5]. Complicated mixtures of organic chemicals are found in herbs and may vary depending upon many factors related to growth production and processing of the herbal product. Though, herbs with anti-microbial, anti-pyretic, anti-inflammatory and immunoreactive properties have been documented[6,7].

Certain chemical compounds such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides which are known as secondary metabolites can be found in the phytogenic plants which are biologically active[8]. They are also known to have high amounts of essential nutrients, vitamins, minerals and fatty acids and fibre[9]. They could be used as feed additives due to their suitability and preference, reduced risk of toxicity and minimize health hazards[10]. Phytogenic feed additives are often claimed to improve the flavour and palatability of feed thus enhancing production performance. However, the number of studies having tested the specific effect of phytogenic products on palatability by applying a choice-feeding design is quite limited. A wide range of spices, herbs and their extracts are known medicine to exert beneficial actions within the digestive tract, such as laxative and spasmolytic effects, as well as prevention from flatulence[11]. A group of researchers found greater enzyme activities in pancreatic homogenates and a pronounced bile acid flow in rats fed those substances[12]. Similarly, essential oils used as feed additives for broilers were shown to enhance the activities of trypsin and amylase[13]. Furthermore, Manzanilla *et al.*[14] fed a combination of essential oils and capsaicin to swine and observed that gastric emptying was slowed down by these additives. Researches shown phytogenic additives could stimulate intestinal secretion of mucus in broilers, an effect that was assumed to impair adhesion of pathogens and thus to contribute to stabilizing the microbial eubiosis in the gut of the animals[15].

African walnut (*Tetracarpidium conophorum*), like many plants in Africa and other parts of the world has been proven to have nutritive, medicinal, agricultural and industrial values over the years. Reports of its phytochemical analysis of showed it contains bioactive compounds such as oxalates, phytates, tannins, saponins and alkaloids which partly show the use of the seeds, leaves and roots in herbal medicine[16]. The presence of tannin supports its anti-inflammatory property[17]. As a rich source of alkaloids, coupled with the presence of the essential vitamins and minerals, *Tetracarpidium conophorum* can be seen as a potential source of useful food and drugs. Its seed is rich in fat, nearly eighty per-cent of polyunsaturated fat with proven cholesterol lowering properties. Studies have shown that *Tetracarpidium conophorum* possess some properties that are required for wound healing like antibacterial and antioxidant activities[18] and immuno stimulating activities[19].

Extracts of *Tetracarpidium conophorum* leaf have been shown to possess good antibacterial activities especially against Gram positive organisms. Results from a study conducted by Onwuli *et al.*[20] suggested that *Tetracarpidium conophorum* nuts have antihyperglycaemic and antimicrobial activities. A research conducted by Okon and Atai[21] discovered that albino wistar rat fed with a standard, commercial diet (control group) or a diet containing *Tetracarpidium conophorum* seeds for 60 days reduced the level of cholesterol and triglyceride in the rats compared with the control group fed with standard diets.

Based on the aforementioned bioactive compounds and properties of (*Tetracarpidium conophorum*) leaves, there is need to know whether (*Tetracarpidium conophorum*) leaf and its extracts could be used as feed additives for meat type chickens for producing safe and cost effective meat. Therefore, the objective of the present study was to evaluate the effect

of African walnut (*Tetracarpidium conophorum*) leaf and its extract on haematology, serum chemistry and serum antioxidant enzymes of broiler chickens.

MATERIALS AND METHODS

Experimental site and research duration: This experiment was carried out at the poultry unit, Federal College of Agriculture, Moor Plantation, Ibadan, Nigeria for duration of 6 weeks (May-June, 2019).

Sourcing and processing of test ingredients: *Tetracarpidium conophorum* leaves were obtained from a farm in Omi Adio, Ibadan. They were washed with clean water and allowed to air dry under shade and thereafter will be ground into powder and stored in the dark in airtight plastic bags at ambient temperature. The proximate analysis of the test ingredient was analyzed in the laboratory according to AOAC[22].

The extractions with acetone and methanol were performed using methods described by Konate *et al.*[23]. The *T. conophorum* leaf extract was obtained from the *T. conophorum* leaf powder a by ethanol as an extracting solvent. The ratio of ethanol volume (mL) and sample weight (g) was 40:1. The leaf powder was soaked for 48 h in ethanol and thereafter sieved. The filtrates were concentrated at a temperature of 50°C using a rotary evaporator and later freeze dried. The leaf extracts were stored in separate dark airtight plastic bags at ambient temperature.

Experimental birds and their management: This study was conducted from 7-49 days of age. A total of 135 day old, unsexed broiler chickens were purchased from a commercial hatchery in Oluyole, Ibadan, Nigeria. Before the arrival of the birds, the brooder house, feeders and drinkers were properly cleaned and disinfected. Wood shavings used as litter materials were spread on the floor of a well heated ventilated brooder house. On arrival, all chickens were fed a commercial broiler starter for 7 days. Then, at 7 days of age, they were individually weighed and randomly distributed into 5 dietary treatments, each treatment included 3 replicates each with a total of 9 birds per replicate on the basis of their weight. Thus, 135 broiler chickens of 7 days of age were used for the experiment. Routine managements, vaccinations and medications were duly followed throughout the course of the study and the experiment lasted for 6 weeks.

Experimental diet: Five experimental diets were formulated with the inclusion of *T. conophorum* Leaf powder (TCLP) and *T. conophorum* leaf extracts (TCLE). The diets were made as followings: (1) T1, control and basal diet, (2) T2, T1+250 mg TCLP (3) T3, T1+500 mg TCLP (4) T4, T1+25 mg TCLE (5) T5, T1+50 mg TCLE kg/diet. The diets were formulated as indicated in Table 1.

Table 1: Composition of the basal diet of broiler chickens

Items	Starter phase 7-21days	Finisher phase 21-49 days
Ingredients (%)		
Maize	58.000	63.000
Soybean meal	37.000	32.000
Limestone	02.050	02.050
Dicalcium phosphate	01.050	01.050
DL-methionine	00.020	00.020
Lysine	00.030	00.030
Mineral premix	00.025	00.025
Salt	00.025	00.025
Total	00.100	00.100
Calculated analysis		
Crude protein	23.0000	20.0000
ME (kcal kg^{-1})	00.3000	00.3200
Lysine (%)	01.0030	01.0000
Methionine (%)	00.0052	00.0038
Calcium (%)	01.0000	00.0090
Phosphorus (%)	00.0080	00.0075

Data collection: At the end of 42 days of study, 2.5 mL of blood was collected from a bird in each replicate making 15 birds through a neck slitting that was collected into labeled sterile universal bottles containing Ethylene-diamine-tetra-acetic acid (EDTA) as anticoagulant and was used to determine the haematological parameters within an hour of sample collection. Other blood samples were collected in non-EDTA tubes and allowed to clot for 1 h, at room temperature and then centrifuged at 3,000 rpm for 20 min, for serum separation. Collected sera were stored in a deep freezer at -20°C until chemically analyzed. At the time of analysis, the samples were thawed and analyzed for total protein, albumin, glucose and total cholesterol.

Haematological parameters and serum parameters: Microhematocrit and colorimetric methods were used to determine the Haemoglobin (g dL^{-1}), packed cell volume (PCV) (%), red blood cells (RBC) (L) and white blood cells (WBC) (L). Serum total protein (g dL^{-1}) was determined colorimetrically, while albumin (g dL^{-1}) value was measured by bromocresol green method. Globulin concentration (g dL^{-1}) was calculated as the difference between total protein and albumin. Total cholesterol (mg dL^{-1}) and high density lipoprotein (HDL) (mg dL^{-1}) were also monitored colorimetrically[23].

Serum antioxidant enzymes: On day 42, the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and contents of malondialdehyde (MDA) were all evaluated according to the spectrophotometrical method described by Hashemipour *et al.*[24].

After centrifuging the samples, SOD and GSHPx activities and MDA levels were assayed by a spectrophotometer .The xanthine oxidase method was utilized for determination of the activity of SOD, which monitors the inhibition of reduction of nitro blue tetrazolium by the sample.

The activity of GSH-Px was determined by 5, 5_- dithiobis-nitrobenzoic acid, using a spectrophotometer at 412 nm. Content of MDA was assayed with 2-TBA and the change of absorbance at 532 nm was monitored by a spectrophotometer

Statistical analysis: All data was analyzed using one way analysis of variance (ANOVA) and means was compared by the Duncan's multiple range test when ANOVA analysis was significant (SPSS 16.0 for Windows, SPSS Inc., Chicago, IL, USA). A p-value of 0.05 or less was declared significant.

RESULTS

Effect of African walnut on hematological parameters of broiler chickens: Table 2 shows the effect of African walnut leaf powder and its extract on haematology parameters of broiler chickens. The results showed that HB, RBC, platelet, lymphocytes, HET (Heterophils) and MON (Monocytes) were significantly ($p<0.05$) influenced by the inclusion of African walnut leaf powder and its extract. The birds fed the control diet (T1) recorded significantly ($p<0.05$) lower value for HB values (7.15) while other treatment groups had comparable values. The highest and lowest value for RBC recorded in birds fed 500 mg TCLP kg/diet (T3) and control diet (T1), respectively ($p<0.05$). The lymphocytes in the blood of the broiler birds fed the control diet (T1) is significantly ($p<0.05$) lower compared to the birds fed 250 mg TCLP kg diet^{-1} (T2). Group fed the control diet (T1) had heterophils value (37.50) higher ($p<0.05$) than other treatment groups. Monocyte value (5.00%) was highest in birds fed 500 mg TCLP kg/diet group while the least value (3.00%) was recorded in both birds fed 250 mg TCLP kg/diet (T2) and 50 mg TCLE kg/diet (T5) except group fed the control diet (T1) and group fed 25 mg TCLE kg/diet (T4) both with monocyte value of (3.50%). Other parameters measured were not significantly ($p<0.05$) influenced by inclusion of African walnut leaf powder and its extract.

Effect of African walnut on serum chemistry of broiler chickens: Table 3 shows the effect of African walnut leaf powder and its extract on serum of broiler chicken. Addition of African walnut leaf powder and its extracts had no significant ($p>0.05$) differences in triglycerides, cholesterol and albumin parameters. High density lipoprotein (HDL) levels of birds from T2-T5 significantly increased ($p<0.05$) than the

Table 2: Effect of African walnut (*Tetracarpidium conophorum*) leaf powder and its extract on haematological parameters of broiler chickens

Parameters	T1	T2	T3	T4	T5	SEM
PCV (%)	23.50	29.50	28.00	26.00	25.00	±0.89
HB (g dL^{-1})	07.15^{b}	09.30^{a}	08.70^{ab}	08.75^{ab}	08.40^{ab}	±0.30
RBC (L)	02.26^{b}	03.32^{ab}	03.42^{a}	02.87^{ab}	02.49^{ab}	±0.17
WBC (×10^{4} L^{-1})	01.50	01.60	01.60	01.50	01.40	±0.03
Platelet (×10^{5})	01.20^{b}	01.40^{a}	01.20^{b}	01.20^{b}	01.30^{ab}	±0.02
Lymphocytes (%)	55.00^{c}	72.00^{a}	63.00^{b}	66.00^{b}	66.00^{b}	±1.52
HET (%)	37.50^{a}	20.00^{c}	29.00^{b}	27.00^{b}	27.00^{b}	±1.55
MON (%)	03.50^{ab}	03.00^{b}	05.00^{a}	03.50^{ab}	03.00^{b}	±0.27
EOS (%)	04.50	05.00	03.00	03.50	05.00	±0.32

Means along the same row with different superscripts are significantly different ($p<0.05$), PCV: Packed cell volume, HB: Haemoglobin, RBC: Red blood cell, WBC: White blood cell, HET: Heterophils, MON: Monocytes, EOS: Eosinophils, T1: Control, T2: 250 mg TCLP, T3: 500 mg TCLP, T4: 25 mg TCLE, T5: 50 mg TCLEs

Table 3: Effect of African walnut (*Tetracarpidium conophorum*) leaf powder and its extract on serum chemistry of broiler chickens

Parameters	T1	T2	T3	T4	T5	SEM
Total protein (g dL^{-1})	03.62^{a}	03.21^{ab}	002.63^{c}	003.05^{bc}	002.86^{bc}	±0.11
Albumin (g dL^{-1})	01.59	01.39	001.37	001.53	001.46	±0.04
Globulin (g dL^{-1})	02.03	01.82	001.26	001.52	001.40	±0.05
Triglycerides (mg dL^{-1})	67.93	59.44	058.96	062.27	091.04	±4.84
HDL (mg dL^{-1})	38.59^{b}	50.77^{a}	044.24^{ab}	048.85^{b}	050.00^{b}	±1.58
Cholesterol (mg dL^{-1})	96.38	95.93	105.43	107.24	101.81	±2.65

Means on the same row having different superscripts were significantly different ($p<0.05$), T1: Control, T2: 250 mg TCLP, T3: 500 mg TCLP, T4: 25 mg TCLE, T5: 50 mg TCLE, HDL: High density lipoprotein

Table 4: Effect of African walnut (*Tetracarpidium conophorum*) leaf powder and its extract on serum antioxidant enzymes of broiler chickens

Parameters	T1	T2	T3	T4	T5	SEM
SOD (U mL^{-1})	18.65^{d}	20.66^{c}	20.45^{c}	25.66^{a}	22.30^{b}	±0.63
GSH-Px (U mL^{-1})	03.44^{d}	04.56^{c}	06.32^{a}	05.65^{b}	04.59^{c}	±0.27
MDA (nmol mL^{-1})	66.80^{a}	46.08^{c}	65.80^{ab}	53.44^{b}	44.15^{c}	±2.55

Means on the same row having different superscripts were significantly different (p<0.05), T1: Control, T2-250 mg TCLP, T3: 500 mg TCLP, T4: 25 mg TCLE, T5: 50 mg TCLE, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, MDA: Malondialdehyde

control group (38.59 mg dL^{-1}). In contrast, the control treatment increased the total protein concentration (3.62 g dL^{-1}) compared to the other treatments (p<0.05).

Effect of African walnut on serum antioxidant enzymes of broiler chickens: Table 4 shows the effect of African walnut leave powder and its extract on serum antioxidant of the broiler chickens. Dietary treatments had impact on SOD and GSH-Px activities and MDA concentrations in the serum. Addition of 25 mg of TCLE significantly (p<0.05) increased the SOD (25.66 U mL^{-1}) of the serum in the broiler chickens while diet T3 (containing 500 mg TCLP) significantly (p<0.05) increased the GSH-Px activities (6.32 U mL^{-1}). Moreover, addition of 50 mg TCLE significantly (p<0.05) decreased the MDA (44.15 nmol mL^{-1}) of the broiler chickens.

DISCUSSION

Haematological parameters are good indicators of the physiological status of birds and its changes are of value in assessing the response of birds to various physiological situations[25]. In the current study, all values of haematology parameters fall in between the levels described by Aiello[26]. The higher (p<0.05) significant value of Hb, RBC, platelet, Lymphocytes, HET and MON indices of the birds on *T. conophorum* leaf powder and its extract relative to the control group is an indication that the birds were not anaemic. There was no significant effect of treatments on the blood concentration of packed cell volume (PCV), WBC and eosinophils (EOS) in broiler. The Hb values at all treatments levels were within the reported range of 9-13 g dL^{-1} with birds fed with 250 mg TCLP kg/diet recording the highest value of 9.30 g dL. The high concentration of Hb in birds fed 250 mg TCLP kg/diet leaf powder might be due to hepato-stimulatory and hepatoprotective effects of leaf meal resulting in the synthesis of more Hb in the bone marrow which is under the control of erythropoietic factors released by hepatic cells[27]. Contrary to the current findings, Chan *et al.*[28] reported that the intake of high dosage of garlic oil significantly increased white blood cell and reduced red blood cell counts and haemoglobin values in rats.

The high HDL recorded in birds fed diet containing 250 mg TCLP showed that *Tetracarpidium conophorum* leaf is a good lipid lowering agent and can reduce incidence of atherosclerosis. This result is in agreement with the findings of Bolukbasi *et al.*[29], who reported that dietary thyme oil increases plasma level of HDL-cholesterol in broiler chickens. This study revealed that an addition of *Tetracarpidium conophorum* had no effect on the selected blood serum biochemical parameters (albumin, globulin, cholesterol and triglyceride).This report is in contrast to the report of Bolukbasi *et al.*[30], who found lower levels of cholesterol and triglycerides as compared with the control group, in the blood of laying hens receiving feed supplemented with a mixture of EO from thyme, sage and rosemary. This result negates the report of 10 who stated that feed supplemented with a mixture of EO from thyme, sage and rosemary lowered blood cholesterol levels and triglycerides in laying hens.

Antioxidant enzymes such as SOD and GSH-Px are one of the defensive mechanisms of the body against the oxidative stress, which protects cells from induced damages caused by reactive oxygen species and reactive nitrogen species[31]. The SOD and GSH-Px enzymes detoxify oxidative stress factors by catalyzing superoxide anions and hydrogen peroxide in the cell[32]. Therefore, superoxide anions are converted to hydrogen peroxide and molecular oxygen by SOD enzyme and then hydrogen peroxide catalyzed to water by GSH-Px enzyme.

In the current study, the use of *Tetracarpidium conophorum* leaf and its extracts increased SOD and GSH-Px activities and reduced MDA contents in the serum. In line with these results, various studies showed that the herbal extracts and probiotic supplements can increase the activity of antioxidant enzymes through enhancing the antioxidant gene expression[33]. Also, this result is in line with the report of Hosseini-Vashan *et al.*[34], who reported an increase in SOD and GSH-Px activity in broilers fed powdered saffron in the diet. Akomolafe *et al.*[35] also reported reduced malondialdehyde level in reproductive organs and accessory glands of rats fed diet containing *Tetracarpidium conophorum* leaves.

The reduced level of MDA resulting from *Tetracarpidium conophorum* leaf and its extracts supplementation implied that it can improve antioxidant

status of animals by activating enzymatic antioxidant system. Therefore, the up-rising application of natural antioxidants in animal feed industries, medical and therapeutics field indicates its potential as promising alternative for synthetic antioxidants owing to its low cost and minimal adverse effects.

CONCLUSION

Natural sources of antioxidants have become a great interest due to their possible usage to replace synthetic ones. Based on the results, it is therefore concluded that broiler chickens fed diet containing 250 mg TCLP increased the HDL cholesterol while all levels of addition of TCLP and TCLE had positive effects on the oxygen carrying capacity of the blood and serum antioxidant enzyme activities. Therefore, *Tetracarpidium conophorum* leaf and its extracts could be potentially used for its antioxidants activity as natural remedy for diseases associated with oxidation stress and further isolation and characterization of the bioactive compounds are encouraged.

SIGNIFICANCE STATEMENT

This study found that the addition of *Tetracarpidium conophorum* leaf powder per kg of diet had positive effects on the high density lipoprotein cholesterol and serum antioxidant enzyme activities of the broiler chickens. This research reveals the role of *Tetracarpidium conophorum* leaf powder and its extracts which contain bioactive compounds such as oxalates, phytates, tannins, saponins and alkaloids in broiler chickens.

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