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Assessment of Bush Buck (*Gongronema latifolia*) as a Dietary Supplement on Haematological and Serum Biochemical Indices of Broiler Chickens

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Abstract

The high cost of conventional feed ingredients for livestock and competition between man and his animals, necessitate the need for alternative and under-utilized plant materials in poultry nutrition. However, in doing so, it is important to ensure that such plant materials are not deleterious to the animals. This research therefore examines the effect of bush buck (*Gongronema latifolia)* leaf extracts as dietary supplements on haematological and serum biochemical parameters of broiler chickens. Fresh leaves (6kg) of bush buck (*Gongronema latifolia)* were after rinsing with de-ionized water air-dried for ten (10) days under room temperature and were ground in a hammer mill to pass through 0.5mm sieve. It was thereafter dissolved in de-ionized warm water (40^{o}C) at 1kg per 5 litres of water and was left to settle for 1 hour before decanting the supernatant. One hundred and ninety-two (192) broiler chicks, after brooding for two weeks were divided into six (6) dietary treatment groups of thirty two (32) birds each. Birds on dietary treatment 1, had no vitamin/mineral supplement in water, while dietary treatment 2 birds had supplemental commercial vitamin/mineral premix (vitalyte extra). Dietary treatments 3, 4, 5 and 6 had *Gongronema latifolia* leaf warm water extract in the following ratios; 25, 50, 75, and 100ml/litre of water respectively. The vitalyte extra was administered (5g/l) once a week while the birds had access to feed and water/GLLE *ad libitum*. On the last day of the experiment, blood samples were collected from the birds for haematological analysis. All the assayed parameters such as packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC), heterophil (H), lymphocyte (L), monocyte (M), eosinophil (E), basophil (B), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were significantly ($P<0.05$) influenced by the dietary treatments. The analyzed serum biochemical indices such as total protein, albumin, globulin, creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase, sodium and potassium were generally better in the 25ml/l GLLE. Glucose was significantly reduced in the vitalyte treatment while total cholesterol, high and low density lipoprotein were least in the 50ml/l GLLE treatment. In conclusion, the results above revealed that the *Gongronema latifolia* leaf warm (40^{o}C) water extract of 50ml/l was safer and supported better health and wellbeing of the broiler chickens. This shows that *Gongronema latifolia* leaf warm (40^{o}C) water extract could be used to wholly replace the conventional vitamins and minerals (vitalyte extra) in broiler diets without any adverse effect on growth and health performance of the birds.

Keywords: *Gongronema latifolia,* broiler chickens, haematological and biochemical indices.

INTRODUCTION

Food supply in most parts of Nigeria is chiefly of starchy grains which supply enough energy but lacking in necessary proteins, vitamins and minerals for body repairs and maintenance. Tewe [1] reported that Nigeria remains amongst the least consumers of animal protein in the world. Continuing, he noted that daily intake of animal protein average was 4.2g per person in contrast to the recommended 35g for proper reproduction, health and survival. To drive home this point, Acholonu [2] reported per caput poultry meat consumption of 38g, 22g, 19g, 18g, 4.8g, 4.8g and 2.2g for USA, Malaysia, Brazil, South Africa, Indonesia, China and Nigeria respectively. This problem of nutritional imbalance is receiving more public attention because of its detrimental effects.

Broiler chickens play an important role in human nutrition because of the protein it contains. Animal production, especially poultry production has contributed considerably to the economic development

of Nigeria. It is one of the profitable agro-industries which can effectively tackle the problem of unemployment in the rural areas. Livestock products accounts for about 30% of the total global value of food and Agriculture, and approximately 19% of the value of global food production [3]. Products from food animal provide over 33% of the protein consumed in human diets globally and about 16% of food energy consumed [4]. Poultry production contributes greatly to the farmer's household. This contribution can be measured in terms of the direct income benefit which plays vital role as a source of readily available cash to the poultry farmers. Obidinma [5] reported that poultry production remains one of the veritable ways of achieving high quality protein to meet the increasing demand of Nigerian teaming populace.

Intensive poultry production was introduced in Nigeria over fifty years ago and has developed rapidly in the last few decades as an important livestock business enterprise in the country [6]. However, the growth and expansion of the industry is confronted by high cost of feed and drugs [7]. Livestock feed costs in developing countries are a continuing challenge hence researches in this area are also continuous. Thus, the high and increasing prices of animal feeds have compelled researchers in developing countries to direct their attention to non-conventional feed ingredients, with particular emphasis on proteins, vitamins and mineral substitutes [8].

Essien *et al.,* [9] noted that the uses of plant extracts in broiler production have been documented. G*ongronema latifolia* commonly called "Utazi" by the Igbos, "Arokeke" by the Yorubas, and "Utasi" by the Efiks and Ibibios respectively, is one of the plants whose extracts (stem, roots and leaves) have been found to be beneficial to both human and animals. G*ongronema latifolia* is consumed fresh, cooked or dried and applied as powdery spice; whichever way, it carries a moderate bitter taste that contributes tremendously to its flavour. The plant leaf extracts have been used as bitter spice or flavoring agent in many traditional dishes which acts as a stimulating tonic for the digestive system. It stimulates the flow of bile and appetite for food and enhances the activities of pancreas, regulates blood sugar and promotes detoxification of the liver [10]. Researchers agree that the fundamental ingredients used for medicinal purposes are stored in the various parts of the plant such as the fruits, leaves, stems, roots and bark. Local poultry farmers use it for the treatment of common respiratory diseases [9]. Gamaniel and Akah [11] reported that the leaf extract contains five phytochemical compounds including alkaloids, saponins, flavonoids, tannins and glycosides and suggested possible varied pharmacological effects. Ajewole [12] noted that leaf vegetables and their extracts are sources of macro and micro nutrients that play a major role in healthy living. He also observed that they are regular ingredients in the diets of the average Nigerian and provides appreciable amount of nutritive minerals and vitamins. Gorge [13], in his submission observed that even though the bulk of the weight is water, leafy vegetables represent a veritable natural pharmacy of minerals, vitamins and phytochemicals. Similarly, the leaf extracts have been shown to have anti-oxidative properties and are being utilized in the management of diabetes mellitus and other tropical diseases of human [14, 15]. Agbo *et al.* [15], identified the crop (Bush buck) to be nutritionally high in iron, zinc, vitamins, proteins and amino acids and thus could complement the inadequacies of these substances in feed.

The development of the potential indigenous plants as sources of animal feedstuff might not only decrease dependency on the industry for expensive imported feed ingredients but relatively reduce the production cost thereby leading to the growers' economic efficiency. This research therefore examines the effect of bush buck (*Gongronema latifolia)* leaf extracts as dietary supplements on haematological and serum biochemical parameters of broiler chickens.

MATERIALS AND METHODS

Experimental design and experimental materials

The experiment was laid out in a Completely Randomized Design (CRD). One hundred and ninety-two (192) broiler chicks after brooding for two weeks were randomly allocated to six experimental treatment diets. Each treatment group was assigned thirty two (32) birds and replicated into four (4) to give eight (8) birds per replicate.

Fresh leaves of *Gongronema latifolia* (Bush buck) were sourced from the Teaching and Research Farm of the University. The leaves were rinsed with clean water to remove dirt and sand and were later air-dried under room temperatures (20-25^{o}C) for 10 days. The dried leaves were milled into powder to pass through 0.5mm sieve and thereafter dissolved in warm water (40^{o}C) at the rate of 1kg in 5 litres. The supernatant after filtering, was stored in a refrigerator that was maintained at 5^{o}C and was used on daily basis as *G. latifolia* leaf extract.

The main feed was commercial broiler feed that was purchased from a commercial animal feed store. The feed was broiler starter and was fed for three (3) weeks and thereafter finisher mash was used for the remaining duration (4 weeks) of the experiment.

Water and the experimental G. *latifolia* leaf extracts (GLLE) were supplied as follows: Treatment 1; Water (W) only with no supplemental multivitamin/mineral drug added; Treatment 2, water and vitalite. Treatments 3, 4, 5 and 6 contained water and GLLE. The vitalyte was administered in water according to the manufacturer's recommendation of 5g

per litre of drinking water while birds in treatments 3, 4, 5 and 6 had theirs in the following proportions; GLLE 25ml, GLLE 50ml, GLLE 75ml and GLLE 100ml per litre of water respectively as shown above. The feed was broiler starter which was fed for three (3) weeks and thereafter finisher mash that was used for the remaining duration (4 weeks) of the experiment.

Haematological and serum biochemistry

On the last day of the feeding trial, blood samples were collected from four broiler chickens per treatment using needles and syringes through the web vein. Samples were collected into a set of sterilized tubes containing ethylenediaminetetraacetic acid (EDTA) labeled bottles as anti-coagulant for haematological determination, while another set of blood samples were collected from the same birds into heparinised tubes for serum biochemical assessment.

Packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and hemobglobin were determined by improved Neubaur after dilution and cyanomethamoglobin methods as described by Dacie and Lewis [16]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and serum metabolites such as total protein, albumin, creatinine and urea were determined by the method of Hyduke [17], while globulin was estimated by the subtraction of albumin value from serum total protein value [16].

Lipid profile and cholesterol determination

Important lipid parameters that were evaluated include serum glucose, total cholesterol, triglyceride, High Density Lipoprotein (HDL-P) and Low Density Lipoprotein (LDL-P) in accordance with AOAC [18].

STATISTICAL ANALYSIS

Data generated were subjected to a one-way analysis of variance (ANOVA) and treatment means with significant differences were compared using Duncan Multiple Range Test as outlined by Steel and Torrie [19] with the aid of SAS [20] package.

RESULTS AND DISCUSSION

Haematological indices

The haematological traits of broiler chickens as influenced by the dietary treatments are depicted in Table 1. The results revealed that all the assayed haematological parameters which included packed cell volume, red blood cell, white blood cell and its differentials, haemoglobin, mean cuspuscular volume and mean cuspuscular haemoglobin were significantly ($P<0.05$) influenced by the dietary treatments. Packed cell volume (PCV) ranged from 26.00% in the 50ml/l GLLE treatment to 30% in the 100ml/l GLLE treatment. The white blood cell (WBC) concentrations were 6.00, 9.20, 7.00, 10.00, 11.30 and 6.80 $x10^{9/L}$ in broilers placed on treatments 1to 6 respectively in that order. On the red blood cell (RBC), the value was highest ($2.83x10^{12/L}$) in treatment 6 of birds given 100ml/l GLLE and least ($2.24x10^{12/L}$) in the 50ml/l GLLE. The RBC close range of 2.24 -$2.88x10^{12/L}$ across treatments, suggests that the tested ingredient successfully and satisfactorily replaced the vitalyte extra in the chickens. The heterophil concentration ranged from 20.00% in treatment 6 with 100% GLLE to 37.00% in treatment 3 with 25% GLLE. The table shows that lymphocyte was significantly highest ($P<0.05$) in treatment 6 (75.00%) and least (58.00%) in the 25ml/l GLLE treatment. All the birds in the treatments had varied levels of monocytes except birds in treatment 2 where it was not detected. The concentration ranged from 1% to 3%. The eosinophil concentration of the experimental birds were as follows; 2.00, 2.00, 3.00, 2.00, 0 and 1% for treatments 1 to 6. It was revealed that eosinophil was significantly highest ($P<0.05$) with 3.00% while treatments 1, 2 and 4 had same value (2.00% each). It was not detected in treatment 5 (75ml/l GLLE). For basophil, treatments 6, 4 and 3 had 1% each while treatments 5 and 2 had 2% each. It was not detected in the water control treatment. Treatment 6 recorded highest value (10g) for haemoglobin followed by birds in treatments 5, 3, 2, 1 and 4 with values 9.60, 9.40, 9.30, 8.60 and 8.40g respectively. Vitamins content of plant food materials like leaves stimulate the synthesis of haemoglobin leading to their increase in the blood Vitamins content of plant food materials like leaves stimulate the synthesis of haemoglobin leading to their increase in the blood. The mean corpuscular volume was highest in treatment 1 with $11.68x10^{5fl}$ and least in treatment 2 with $10.37x10^{5fl}$. Values for mean corpuscular haemoglobin were close. It ranged from 33.02 - 35.34pg. The highest value was observed in treatment 6 with 35.34pg followed by treatments 2, 5, 1, 3 and 4 with their corresponding values being 34.30, 34.28, 34.26, 33.57 and 33.02pg respectively. Mean corpuscular haemoglobin concentration (MCHC) was also significantly ($P<0.05$) affected by the treatments in all the birds. It ranged from 31.85g/dl in treatment 1 to 34.42g/dl in the 75ml/L GLLE birds. As shown above there is a close range in the values of MCH, MCV, basophils, eosinophils and monocytes of the chickens which may give credence that the GLLE is a viable option for the vitalyte extra, a commercial multivitamin supplement for poultry in Nigeria.

Table 1. Assessment of Bush Buck (G*ongronema latifolia*) as a dietary supplement on haematological indices of broiler chickens

Parameters	T1 Water	T2 Vitalyte Extra	T3 25ml/l GLLE	T4 50ml/l GLLE	T5 75ml/l GLLE	T6 100ml/l GLLE	SEM (±)
	Dietary Treatments						
Packed cell volume (%)	27.00^{bc}	28.00^{b}	28.00^{b}	26.00^{c}	28.00^{b}	30.00^{a}	0.57
Red blood cell (x$10^{12/L}$)	2.51^{e}	2.70^{c}	2.68^{d}	2.24^{f}	2.74^{b}	2.83^{a}	0.19
White blood cell (x$10^{9/L)}$	6.00^{e}	9.20^{c}	7.00^{d}	10.00^{b}	11.00^{a}	6.80^{de}	0.25
Heterophil (%)	30.00^{c}	28.00^{d}	37.00^{a}	31.00^{b}	23.00^{e}	20.00^{f}	0.65
Lymphocyte (%)	67.00^{cd}	68.00^{c}	58.00^{d}	66.00^{e}	73.00^{b}	75.00^{a}	0.40
Monocyte (%)	1.00^{c}	0.00^{d}	1.00^{c}	3.00^{a}	2.00^{b}	3.00^{a}	0.14
Eosinophil (%)	2.00^{b}	2.00^{b}	3.00^{a}	2.00^{b}	0.00^{d}	1.00^{c}	0.17
Basophil (%)	0.00^{c}	2.00^{a}	1.00^{b}	1.00^{b}	2.00^{a}	1.00^{b}	0.26
Haemoglobin (g/dl)	8.60^{b}	9.30^{ab}	9.40^{ab}	8.40^{bc}	9.60^{a}	10.00^{a}	0.37
Mean corp. volume(x10^{5fl})	11.86^{a}	10.37^{b}	10.44^{b}	10.50^{b}	11.60^{a}	10.67^{b}	0.40
Mean corp. haemoglobin(pg)	34.26^{a}	34.40^{a}	33.57^{b}	33.02^{bc}	34.28^{a}	35.34^{a}	0.46
Mean corp. haemoglobin con.(g/dl)	31.85^{c}	33.21^{b}	33.57^{b}	32.30^{c}	34.42^{a}	33.33^{b}	0.33

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

Table-2: Assessment of Bush Buck (*Gongronema latifolia*) as a dietary supplement on Serum biochemical indices of broiler chickens

Parameters	T1 Water	T2 Vitalyte Extra	T3 25ml/l GLLE	T4 50ml/l GLLE	T5 75ml/l GLLE	T6 100ml/l GLLE	SEM (±)
	Dietary Treatments						
Protein (g/l)	67.70^{b}	57.00^{d}	73.00^{a}	59.50^{c}	61.40^{c}	61.70^{c}	2.48
Albumin (g/l)	39.50^{b}	32.80^{c}	41.60^{a}	32.50^{c}	39.00^{b}	40.40^{b}	0.77
Globulin (g/l)	28.20^{b}	24.20^{d}	31.40^{a}	27.00^{c}	22.40^{e}	21.30^{f}	0.48
Creatinine (mg/dl)	0.90^{d}	1.00^{bc}	0.70^{e}	1.30^{b}	1.00^{bc}	1.40^{a}	0.09
Uric acid (mg/dl)	7.10^{a}	6.50^{b}	2.00^{d}	5.90^{bc}	5.40^{bc}	5.00^{c}	0.27
AST. (iu/l)	120.40^{d}	116.80^{e}	163.90^{a}	141.00^{b}	128.50^{c}	97.50^{f}	0.89
ALT. (iu/l)	25.60^{c}	17.50^{d}	34.70^{a}	10.50^{f}	26.40^{b}	16.90^{e}	0.32
Calcium (mg/dl)	10.30^{a}	11.20^{a}	14.00^{a}	12.50^{a}	16.30^{a}	11.40^{a}	0.39
Sodium (mg/dl)	7.60^{b}	4.60^{e}	4.30^{f}	8.30^{a}	6.50^{d}	7.10^{c}	0.11
Potassium (mg/dl)	121.00^{c}	115.80^{d}	120.30^{b}	109.40^{e}	105.30^{f}	130.00^{a}	0.14

GGLE - ***Gongronema latifolia*** leaf extract.

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

Serum biochemical indices

The serum biochemical parameters as influenced by the dietary treatments (Table 2) revealed significant (P<0.05) differences on the total protein, albumin, globulin, uric acid, calcium, sodium, potassium, aspartate aminotransferase, alanine aminotransferase and creatinine. The use of chemical indices as a pointer to conditions that cannot be readily and physically noticed by performance indices cannot be overemphasized. Hofferberg and Block [21] reported that the serum protein, albumin and globulin synthesis is related to the availability of protein and micro-nutrients. The result of this work shows an indefinite trend on the serum biochemical of the broiler chickens. For instance, total protein had the following values; 67.70, 57.00, 73.00, 59.50, 61.40 and 61.70g/l respectively for treatments 1- 6. As observed above, total protein was highest in treatment 3, lowest in treatment 2 and slightly higher in treatment 4, so there is an indefinite direction. The albumin, globulin, AST and ALT of treatment 3 with 25ml/l GLLE were also higher in values than other treatments. Ibrahim and El-Bahr (2012) reported a similar range of aspartate aminotransferace (AST) but there were comparatively lower values of alanine aminotransferace (ALT). In their findings, total protein, albumin and globulin values were also lower than the report of this research. There were close range of values in uric acid. In birds, uric acid is the major nitrogen metabolic end product whose concentration is influenced by many factors such as age, diet and laying period [22]. Creatinine, the biomarker of protein metabolism is normally low in birds and its high level is associated with high level of activity [23]. The values determined in this study were of the same range with the report of Ibrahim and El-Bahr [24]. The concentration of calcium was highest (16.30mg/dl) in treatment 5 with the composition of 75ml/l GLLE. Treatment 6 with 100ml/l GLLE had the highest concentration of potassium but lowest in birds fed 75ml/l GLLE (treatment 5). The lack of definite trend and inconsistency are in tandem with the results (findings) of Antai *et al*. [25] and Akinnuga *et al.* [26]. In the report of Attia *et al*. [27], there were no significant differences (P>0.05) on the serum parameters of the broilers on the tested extracts. This contradicts the report of this research. They also reported no deleterious effect on the health of the birds as was corroborated by this research. The result of this research may also support the local assumption that *Gongronema latifolia* has a cleansing effect on the body of the animal.

Table-3: Assessment of Bush Buck (*Gongronema latifolia*) as a dietary supplement on glucose and lipid profile of broiler chickens.

	Dietary Treatments						
Parameters	T1 Water	T2 Vitalyte Extra	T3 25ml/l GLLE	T4 50ml/l GLLE	T5 75ml/l GLLE	T6 100ml/l GLLE	SEM (±)
Glucose mg/(dl)	94.40^{c}	84.50^{f}	126.60^{a}	87.50^{e}	95.50^{b}	93.00^{d}	0.63
Cholesterol mg/(dl)	176.40^{b}	110.90^{e}	182.50^{a}	106.60^{f}	150.20^{c}	116.20^{d}	0.38
Triglycerol mg/(dl)	109.70^{a}	71.80^{f}	99.40^{b}	88.40^{c}	74.60^{e}	84.90^{d}	0.43
High density Lipoprotein (mg/dl)	34.80^{b}	32.80^{c}	26.80^{d}	22.80^{e}	38.40^{a}	21.60^{e}	0.59
Low density Lipoprotein (mg/dl)	110.70^{b}	109.70^{c}	132.80^{a}	53.30^{f}	63.30^{e}	77.60^{d}	1.03

GGLE - G*ongronema latifolia* leaf extract.

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

Glucose and Lipid Profile

As shown in Table 3, the assayed lipid profile of broiler chickens as influenced by the dietary treatments were significant (P<0.05). Crouse and Grurdy [28] reported that the liver had a central role on the maintenance of lipid homeostasis and presence of toxicants may alter the concentration of serum lipids which could increase the risk of atherosclerosis and related cardiovascular diseases. In this report, birds on treatment 3 with 25ml/l GLLE recorded the highest value in serum glucose concentration while the vitalyte diet had the least. The 50ml/l treatment also had a significantly decreased level being next to the vitalyte treatment. The fact that the 50ml/l treatment birds had significantly reduced total cholesterol, triglycerides, low and high density lipoprotein suggests the superiority of the birds compared to others. In conclusion, the results above revealed that the *Gongronema latifolia* leaf warm (40^{o}C) water extract of 50ml/l was safer and supported better health and wellbeing of the broiler chickens. This shows that *Gongronema latifolia* leaf warm (40^{o}C) water extract could be used to wholly replace the conventional vitamins and minerals (vitalyte extra) in broiler nutrition without any adverse effect on health of the birds.

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