Nigerian J. Anim. Sci. 2018, 20 (2): 211-221

Nutritional Evaluation of Bitter Leaf meal (*Vernonia amygdalina*): Effects on Performance, Carcass and Serum Metabolites of Broiler Chickens

Daramola [1], O.T , Oloruntola [2], O.D. and Ayodele [1], S.O.

[1]*Department of Agricultural Technology, The Federal Polytechnic, Ado-Ekiti, P.M.B 5351, Ado-Ekiti, Ekiti-State.*
[2] *Department of Animal Science, Adekunle Ajasin University, Akungba Akoko, Ondo State*

Corresponding Author: *olajumoke.daramola2016@ gmail.com 08060358996*

Target Audience: *Animal Nutritionist, Poultry Farmers and Researchers*

Abstract

A study was conducted to evaluate the phytochemicals present in bitter leaf meal and to determine the effect of graded levels of bitter leaf meal (BLM) on performance, carcass quality, internal organs and serum metabolites of broiler chickens. A total of 72 one-day-old broiler chicks of Abor-acre breed were used for the trial and fed with diets supplemented with graded levels of bitter leaf meal (0%, 0.1%, 0.2% and 0.3%). The diets were isonitrogenous and iso-caloric. The chicks were grouped into 4 treatments (18 chicks per treatment) and teach treatment was replicated 3 times using a complete randomized design. The experiment lasted for 42 days. Results showed a relatively high proximate and mineral content while phytochemical screening revealed that BLM contains bioactive substances beneficial to broiler chickens with anti-nutrient concentrations below the critical levels. The final liveweight, carcass weight and dressing percentage at 1362.27g, 1138.85g and 75.22%, respectively improved in treated groups especially among birds fed diet containing 0.3% BLM. The improvement in the feed conversion ratio (FCR) at 2.56 resulted in improved muscle development. The inclusion of BLM at 0.3% reduced the level of cholesterol, triglyceride, glucose, low density lipoprotein, creatinine at 70.67mg/dl, 34.67mg/dl, 10.33mmol/l , 7.67mg/dl and 0.12mg/dl, respectively, and increased the high density lipoprotein values at 90.33mg/dl. It was concluded that BLM is not deleterious to the growth and health status of broiler chicken rather it can be a good growth booster.

Keywords : *broiler chickens, bitter leaf meal, growth performance, serum metabolites.*

Description of the problem

Herbs are plants that contain substances that are precursors for the synthesis of useful drugs (1). There are some herbs that are safe and others containing cardioactive glycosides which can only be consumed in small quantities but which at such dosage are suitable for the treatments of cardiovascular diseases (2). The use of herbs as dietary supplement substituting synthetic antibiotics in animal production is increasing (3). This is because consumers are rejecting the use of synthetic chemicals such as those used in animal production; their use is associated with human and animal health risk (4 and 5). However, animal producers are aware of market failure due to the ban on the use of antibiotic growth promoters (6 and 7).

Due to these reasons, the search for alternatives to synthetic growth promoters began several years ago and various alternatives have been proposed that yield similar benefits e.g the use of medicinal plant and herbs Recently, attention had shifted to the effect of herbal supplement on poultry due to complexity of variability of bioactive compound including the use of bitter leaf.

Supplementation of herbs into diets of

poultry as phytogenetic feed additive is to exert antimicrobial properties against pathogens, improve bird performance and health status.

Bitterleaf (*Vernonia amygdalina*) leaf contains bioactive compounds (vernolide and vernodalol), which are responsible for its various phytochemicals including flavonoid, alkaloids and phenolics (8). Similarly, it is reported to have antibacterial, antiviral, anti-inflammatory and antilipidaemia (8). This study was therefore designed to determine the benefits on performance, carcass quality, internal organs and serum metabolites of broiler chicken.

Materials and methods

Processing of bitter leaf meal and diet formulation

Bitter leaf was harvested fresh from maturing stems found growing within the Federal Polytechnic Ado Ekiti, Ekiti State Nigeria. The fresh leaves were immediately subjected to air drying for 7 days in an open cleaned concrete floor space until moisture content became constant at 13%. The air-dried leaves were later milled using a commercial feed milling machine. The proximate analysis, phytochemical and mineral content were assessed and thereafter, the bitter leaf meal (BLM) was used to formulate diets along with other ingredients purchased locally. Four diets were formulated such that diet1 was the control diet with no BLM inclusion. Diets 2, 3 and 4 were formulated to contain 0.1, 0.2 and 0.3% BLM respectively as a feed additive. All the diets were compounded to be iso-nitrogenous and iso-caloric. The experimental diets are presented in Tables 1and 2 for the starter and finisher phases respectively.

Table 1: Composition of experimental diets (g/100g) for broiler starter

	Diets bitter leaf meal			
Ingredients	1 (control)	2 (0.1%)	3(0.2%)	4(0.3%)
Maize	43.50	43.50	43.50	43.50
Groundnut cake	16.00	16.00	16.00	16.00
Soyabean	25.00	25.00	25.00	25.00
Wheat offal	6.50	6.50	6.50	6.50
Fishmeal (72% CP)	2.00	2.00	2.00	2.00
Bone meal	3.00	3.00	3.00	3.00
Oyster shell	3.00	3.00	3.00	3.00
NaCl	0.20	0.20	0.20	0.20
Methionine	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Premix	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00
Calculated composition				
Crude protein (g/100g)	23.27	23.27	23.27	23.27
Crude fibre (g/100g)	3.87	3.87	3.87	3.87
ME (Kcal/kg) Phosphorus	2732.9	2732.9	2732.8	2732.8
Calcium	0.78	0.78	0.78	0.78
Lysine	2.30	2.30	2.30	2.30
Methionine	1.39	0.66	1.39	1.39
	0.66		0.66	0.66

Vitamin A15,000I.U., vitamin D_3 13000 I.U, thiamine 2.0mg, riboflavin 6.0mg, pyridoxine 4.0mg, Niacin, 40mg, cobalamine 0.05mg,biotin 0.08mg panthemic acid 5.0mg, folic acid 0.5mg, biotin 0.08mg, cholinechloride 0.05g, manganese 0.096g, zinc 0.06g, coper 0.006g, iodine 0.0014g, selenium 0.24g cobalt 0.25g and antioxidant 0.125g.

Table 2: Composition of experimental diets (g/100g) for broiler finisher

	Diets			
	1	2	3	4
		bitter leaf meal inclusion		
Ingredients	control	0.1%	0.2%	0.3%
Maize	50.00	50.00	50.00	50.00
Groundnut cake	13.00	13.00	13.00	13.00
Soyabean meal meal	20.00	20.00	20.00	20.00
Wheat offal	6.00	6.00	6.00	6.00
Fishmeal (72% CP)	2.00	2.00	2.00	2.00
Palm oil	2.00	2.00	2.00	2.00
Bone meal	3.00	3.00	3.00	3.00
Oyster shell	3.00	3.00	3.00	3.00
NaCl	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20
Premix	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00
Calculated composition				
Crude protein (g/100g)	21.04	21.04	21.04	21.04
Crude fibre (g/100g)	3.48	3.48	3.48	3.48
M/energy (Kcal/kg)	2908.16	2908.16	2908.1	2908.1
Phosphorus	0.74	0.74	0.74	0.74
Calcium	2.36	2.36	2.36	2.36
Lysine	1.02	1.02	1.02	1.02
Methionine	0.50	0.50	0.50	0.50

Vitamin A 10,000I.U., vitamin D 12000 I.U, vitamin E 20I.U, vitamin K 2.5mg, thiamine 2.0mg, riboflavin 3.0mg, pyridoxine 4.0mg, Niacin, 20mg, cobalamin, 0.05mg, panthemic acid 5.0mg, folic acid 0.5mg, biotin 0.08mg, choline chloride 0.2mg, manganese 0.006g, zinc 0.03g, coper 0.006g, iodine 0.0014g, selenium 0.24g cobalt 0.25g and antioxidant 0.125g.

Management of experimental birds and experimental design

The study was performed with 72day-old Arbor-acre strain. The birds were divided into 4 groups of 18 birds each. Each treatments group was further subdivided into 3 replicates of 6birds in a Completely Randomised Design (CRD) such that the mean group weights were similar at the beginning of the experiment. Water was provided *ad-libitum* and glucose as an antistress factor. The birds were given all necessary medications and vaccinations as at when due. The chicks were fed the experimental diet for 42days during which records on daily feed consumption and body weight gain were recorded.

Proximate composition, phytochemical and mineral content determination

Proximate composition of the BLM was determined as outlined by AOAC (9) while flavonoid was determined using (10) method, phytate was determined using (11) method, oxalate content was determined using the method of (12) while phenol was determined by spectrophotometric method. The sodium and potassium contents were determined by flame photometry. The other mineral elements

were determined after wet digestion with a mixture of nitric, sulphuric and hydrochloric acid using Atomic Absorption Spectrophotometer (AAS model SP9).

Performance, carcass and organ measurements

Data on growth performance of the experimental bird were obtained from the daily weekly feed intake and weekly body weight records. These were used to calculate the feed conversion ratio at the end of the experiment. At the end of the experiment, the two birds were selected from each replicate fasted and weighed prior to slaughtering. The carcass weight, dressed weight and weight of the organs were obtained. The weight of the dressed weight and organs were expressed as the relative percentage of the liveweight.

Blood collection for analysis

At the end of the study, prior to blood collection, the birds were starved of feed, 3 birds per treatment were randomly selected and bled by severing the jugular vein. About 5ml of blood samples were collected from each bird in labelled vacutainer tubes without anticoagulants and taken to the medical laboratory for analysis. The serum metabolites such as total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein was calculated. Glucose, creatinine and total protein were determined.

Data collection and statistical analysis

The data were analysed statistically using one-way analysis of variance (ANOVA). Significant differences were separated by Duncan's Multiple Range Test.

Results and Discussion

Proximate composition, mineral contents and phytochemicals

The result of proximate composition, mineral contents and phytochemicals are shown in Table 3. The moisture content of BLM was 10.78±1.05%. This value was higher than that reported (10.02%) by (13).The variation may be due to soil nutrients and environmental factors which have impact on the nutrient availabilities for plants. It was reported (14) that the high moisture content provides greater activity of water-soluble enzymes and co-enzymes needed for metabolic activities of these leafy vegetables. The crude protein content (15.60±1.58%) was higher than that reported for some leafy vegetables such as *Momordica balsamina* (11.29%) and lower than *Amaranthus cruentus* leaf meal (23.0±0.55%). (15) noted that food plants provide more than 12% of their calorific value from protein. The fat content (5.00±0.10%) indicated the presence of oil in bitter leaf. The value recorded was higher than that reported for some other leafy vegetable such as *Ocimum viride* (16). The crude fibre content was found to be (10.58±0.18%), this value fell within the range (8.50-20.90%) for some Nigerian vegetables (17).The ash content was (5.57±0.21%) which, was however lower than that reported by (13) as 9.56% for bitter leaf. The presence of ash content in bitter leaf is a confirmation of the availability of mineral elements. The nitrogen free extract content (52.12±0.47%) was higher than that reported by some researchers.

The sodium content in bitter leaf would help in the regulation of plasma volume, acid base balance, nerve and muscle contractions (18). The value of sodium content of bitterleaf was 7229.58±20.56mg/kg which is higher than the value reported by (13) for the same plant. The calcium and potassium contents were higher in values when compared to the values reported by (13). The zinc content (34.27±2.55mg/kg) was higher than that reported by (19). Zinc is an important micronutrient essential for development and immune functions (20). The iron content is high (68.89±1.91mg/kg) and this plays an

important function in haemopoiesic, control of infection and cell mediated immunity (21).

Phytochemicals are not a necessity for normal metabolism and their absence will not result in a deficiency disease and they are not required for the functioning of the body but they are of benefit to health and play an active role in the treatment of diseases. The flavonoid content was 4.84±0.16mg/100g, phytate 17.53±0.15mg/100mg, oxalate was 3.76±0.05mg/100g and phenol was 1.90±0.17mg/100g. Flavonoids have been found useful in the formulation of drugs, in food, feed, drinks and beverage industries. Flavonoids including phenolic acids showed inhibitory activity towards bacteria (22).

Table 3. Proximate analysis, mineral analysis and phytochemicals in bitterleaf meal (BLM).

Proximate content (%)		Mineral content (mg/kg)		Phytochemicals (mg/100g)	
MC	10.78±1.58	Na	7229.58±20.56	flavonoid	4.84±0.16
CP	15.60±0.17	Ca	1664.52±31.83	Phytate	17.53±0.15
CF	10.58±0.18	K	8722.21±214.92	Oxalate	3.76±0.05
EE	5.00±0.10	Zn	34.27±2.55	Phenol	1.90±0.17
Ash	5.57±0.21	Fe	67.54±1.91		
NFE	52.12±0.47				

MC moisture content; CP crude protein; CF crude fibre; EE ether extract; NFE; Nitrogen free extract; Na sodium; Ca calcium; K potassium; Zn zinc; Fe iron

Table 4 shows the performance characteristics of broiler chickens fed the experimental diets. The average body weight gain (BWG) for the experimental period of 42 days indicated that birds on experimental diet were not significantly ($P>0.05$) influenced by dietary treatments. The feed conversion ratio (FCR) of birds on diets 3 and 4 were similar ($P>0.05$) but significantly lower ($P<0.05$) than that of birds on the control diet. It is noteworthy that FCR values recorded for bird fed diet 4 had the best growth performance when compared to other treated groups and the control. The final liveweight, body weight gain and FCR of birds on 2% bitterleaf meal treatment were 1362.27g, 32.44g and 2.56g, respectively. These are in tandem with the report of (23) and (24), in which they reported improved growth performance of birds fed bitter leaf meal. The improvement observed in this study shows that there is correlation between the body weight gain and lower feed conversion ratio observed in the treated group. The lower the feed conversion ratio the better. The inclusion of bitter leaf meal in cockerel feed significantly improved FCR (25). (26) reported that bitter leaf enhanced gastro intestinal enzymes (chymotrypsin) production which may

improve not only the utilization of feed but could aid in the digestion of sporozites and other intestinal parasites that could reduce feed utilization. (27) opined that the improvement observed in performance of birds fed with bitter leaf meal could be associated with the beneficial effect of bitter leaf in enhancing the gastro intestinal enzyme thereby improving digestion and assimilation of nutrients. The result of this study disagrees with the report of (28) who observed that inclusion of bitter leaf as a feed additive did not improve weight gain and FCR in broilers.

Table 4: Effect of bitter leaf meal on growth performance of broiler chicken (0-42day)

	Treatments				
	1	2	3	4	SEM
	Bitter leaf meal inclusion				
Parameters	(0%)	(0.1%)	(0.2%)	(0.3%)	
Initial body weight (g)	35.92	36.89	35.68	35.66	0.24
Final liveweight (g)	1322.00	1282.57	1326.62	1362.27	13.36
Body weight gain (g)	31.48	30.54	31.59	32.44	0.32
Feed intake (g)	92.75^{a}	84.14^{ab}	84.36^{ab}	83.03^{b}	1.65
Feed conversion ratio	2.94^{a}	2.75^{ab}	2.67^{b}	2.56^{b}	0.04

Means with different superscript on the same row differ significantly ($P<0.05$)

Table 5 presents data on carcass quality and internal organs. The liveweight, carcass weight and dressing percentage improved in treated group especially in diet containing 3% inclusion. However, T3 (2%) and T4 (3%) had higher values of liveweight and carcass weight when compared with T1 (0%) and T2 (1%). The results of the present study conforms with the report of (29) and (30) that dietary supplementation of broilers with phytochemicals improved quality parameters including carcass weight, dressing percentage and reduced abdominal fat. (31) opined that there were no changes in these parameters. The values of bile, spleen and heart of birds on 0.3% bitterleaf meal diet (T4) were lower than the values obtained from chickens on the control diet (0%) and other treatment groups indicating that inclusion of bitter leaf meal at the present level may not cause any detrimental effect on the organs of the birds but the lower values recorded for birds on 0.3% may reflect the effect of high level of bitterleaf meal on the organs which reduces the weights of the organs.

Table 5: Effect of bitter leaf meal on carcass quality and internal organs of broiler chicken (0-42day)

	Treatments				
	1	2	3	4	SEM
	Bitter leaf meal inclusion				
Parameters	(0%)	(0.1%)	(0.2%)	(0.3%)	
Live weight (g)	1254.10^{b}	1284.23^{b}	1327.65^{ab}	1376.15^{a}	16.97
Carcass weight (g)	1033.05^{b}	1063.10^{b}	1096.30^{ab}	1138.85^{a}	14.80
Dressing percentage (%)	73.64^{ab}	72.92^{b}	73.65^{ab}	75.22^{a}	0.37
Liver (%)	32.41	32.63	32.79	34.07	0.67
Kidney(%)	9.75	11.04	10.05	11.66	0.37
Lung(%)	9.02	9.17	8.76	8.88	0.12
Gizzard(%)	45.44	42.97	46.74	45.19	0.96
Bile(%)	3.14^{a}	2.72^{ab}	2.92^{ab}	2.55^{b}	0.09
Spleen(%)	4.66^{a}	4.28^{ab}	4.15^{b}	4.02^{b}	0.10
Heart(%)	6.07^{a}	6.07^{a}	5.25^{b}	5.76^{ab}	0.14

Means with different superscript on the same row differ significantly (P<0.05) .Expressed as percentage of live weight.

The effect of varying levels of bitter leaf meal as feed additive on the serum metabolites of broiler chicken is shown in Table 6. The serum concentration of cholesterol was significantly (P<0.05) higher for birds on control diet when compared to the treated groups. The cholesterol levels of birds on diets supplemented with bitter leaf meal at 1%, 2% and 3% were reduced by 13.31%, 15.47% and 23.74%, respectively. Also there existed significant (P<0.05) higher concentrations of triglyceride and glucose in the control group. The percentage reduction in triglyceride levels were 8.74%, 25.51% and 30.20% while in glucose were 19.78 %, 20.96% and 23.48%, respectively for treatments 2 (1%), 3(2%), and 4(3%). The high density lipoprotein of birds on treatments 3 and 4 were similar (P>0.05) but significantly (P<0.05) higher than that of birds on the control diet (0%) and treatment 2. The low density lipoprotein and creatinine levels of birds on T1 and T2 were similar (P>0.05) but significantly (P<0.05) higher than the levels of birds on T3 and T4.Total protein levels of birds on control diet was significantly (P<0.05) higher when compared to other treatment groups.

Total cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL) serve a diagnostics indices in conditions such as chronicle jaundice, coronary heart disease and atherosclerosis. Hyperlipidaemia is one of the risk factor for cardiovascular disease while cholesterol is the major lipid constituent of atherosclerotic plaque (32). The use of bitter leaf meal significantly lowered the cholesterol and triglyceride levels of the birds indicating that the use of the plant did not contribute to any disease associated with artherosclerosis. The elevated levels of triglyceride and cholesterol obtained in birds on control diet may be as a result of increased level of serum lipids which is related to increase oxidative damage which affects antioxidant status and lipoprotein levels (33 and 34). Inclusion of bitter leaf meal in broiler chicken diets increased high density lipoprotein (HDL), indicating its protective

role against cardiovascular diseases. The protective roles of HDL from cardiovascular disease have been suggested to occur in various ways (35). HDL exerts part of its anti-atherogenic effect by hindering low density lipoprotein (LDL) oxidation. Inclusion of bitter leaf meal at 1%, 2% and 3% significantly lowered low density lipoprotein levels. LDL may be used in monitoring the treatments of patients with elevated blood cholesterol levels (27). In this study, bitter leaf meal elicited beneficial effects by lowering the levels of LDL of the broiler chickens. The creatinine and total protein levels of treated groups were lower when compared to control diet. (36) reported that decreased values of creatinine are not clinically significant. The test ingredient may not have effect on the kidney since creatinine levels were low. The decrease in total protein level may be due to decrease in feed intake or absorption as a result of bitterness of the leaves.

Table 6: Lipid profile and serum metabolites of broiler chicken fed varying levels of bitterleaf meal (0-42day)

	Treatments				
	1	2	3	4	SEM
	Bitter leaf meal inclusion				
	(0%)	(0.1%)	(0.2%)	(0.3%)	
Parameters					
Cholesterol (mg/dl)	92.67^{a}	80.33^{ab}	78.33^{ab}	70.67^{b}	3.14
Cholesterol reduction (%)	0	13.31	15.47	23.74	-
Triglyceride (mg/dl)	49.67^{a}	45.33^{a}	37.00^{b}	34.67^{b}	2.11
Triglyceride reduction (%)	0	8.74	25.51	30.20	-
Glucose (mmol/L)	13.50^{a}	10.83^{b}	10.67^{b}	10.33^{b}	0.47
Glucose reduction (%)	0	19.78	20.96	23.48	-
HDL(mg/dl)	75.00^{b}	76.00^{b}	87.33^{a}	90.33^{a}	2.20
LDL (mg/dl)	13.00^{a}	10.67^{a}	7.80^{b}	7.67^{b}	0.75
Creatinine (mg/dl)	0.19^{a}	0.17^{a}	0.13^{b}	0.12^{b}	0.01
Total protein (g/d)	29.67^{a}	27.17^{b}	25.90^{bc}	23.83^{c}	0.71

Means with different superscript on the same row differ significantly (P<0.05) . HDL-High density lipoprotein; LDL- low density lipoprotein.

Conclusions and Applications

1. It can be concluded that bitter leaf meal (BLM) enhanced the gastro intestinal enzyme thereby improving digestion and assimilation of nutrients.
2. Inclusion of BLM at 0.3% improved carcass quality parameters such as carcass weight, dressing percentage and has no deleterious effect on the internal organs of the birds
3. It was observed that inclusion of BLM at 0.3% positively influenced the serum metabolites, thereby confirming that BLM can reduce the risk factors of high cholesterol level.

References

1. Sofowora, E.A (2008). Medicinal plants and traditional medicine in Africa. 3rd Edn., Spectrum Books Limited Ibadan, Nigeria, pp. 199-204.
2. Mohammed, A. (2008).Pharmacognosy: pharmacognosy aqueous leaf extract of *Vernonia amygdalina* on lipoprotein and oxidative status in diabetic rat model. Nigeria *Journal of Physiological Science.* 20 (12); 30-42.
3. Akanmu, M.A and Adeyemo, G.O (2012). The effect of neem and pawpaw leaves supplementation on blood profile of broilers. *International Journal of Current Research* 4 (12): 272-276.
4. Hao, H., Cheng, G., Iqbal, Z., AI, X., Hussan, H.L., Huang, L., Dai,M., Wang, Y., Liu, Z.and Yuan, Z.H (2014). Benefit and risks of antimicrobial use in food producing animals. *Journal of Food Microbiology*. 5 (288): 1-11.
5. Gonzalel, R.M. and Angels, H.J.C (2017). Antibiotic and synthetic growth in animal diets : Review of impact and analytical methods. Food Control 72: 255-267.
6. EC. (2003). Regulation EC. No 1831/2003 of the European Parliament and Council of 22 September, 2003 on Additives for Use in Animal Nutrition. Off J. Eur. Commun. L 268 : 29-43.
7. FAO/OIE/WHO. (2004). Second join FAO/ OIE/WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: management options. Oslo.
8. Sani, A.A., Alemika, E.T., Abdulraheem, R.O .,Abdulkareem, S.S., Abdulraheem, R.B and Ilyas, M. (2012). A study review of documented phytochemistry of *Vernonia amygdalina* (Family Asteraceae) as the basis for pharmacologic activity of plant extract. *Journal of Natural Science Resources* 2 (7) pp.1-8.
9. AOAC. (1995). Association of Official Analytical Chemistry, Official Methods of Analysis 16th edition, Washington D.C.
10. Boham, B.A. and A.C Kopcipai, (1974). Flavonoids and condensed tannins from leaves of Havairan vaccinium and calycinium. Pacific Sci. 48: 458-463.
11. Rahila, I. Rukhdandra, H., Zaidi, A.A and Shanusshalia, R. (1994). Phytochemical screening of medicinal plants belonging to Euphobiacae *Pakinstan Veterinary Journal*. 14: 160-162.
12. A.O.A.C.(2005). Official methods of Analysis of Association of Analytical Chemists. Washington D.C
13. Asaolu, S.S., Adefemi, O.S., Oyakilome, I.G., Ajibulu, K.E and Asaolu, M.F(2012). Proximate and mineral composition of Nigerian Leafy Vegetable. *Journal of Food Research,* 1(3): 214-218.
14. Iheanacho, K. and Ubebani, A.C (2009). Nutritional composition of some leafy vegetable consumed in Imo-State, Nigeria. *Journal of Applied Science Environmental Management* 13 (3): 35-38.
15. Ali, A. (2009). Proximate and mineral composition of the marchubeh (*Asparagus officinalis*). World Dairy and Food Science, 4(2). 142-149.
16. Udosen, E.O.(1995).Proximate and mineral composition of some Nigerian Vegetables. *Discovery Innovations*, 7: 383-386.
17. Isong, E. and Idiong. (1997). Nutrient content of the edible leaves of seven wild plants from Nigerian. *Plant Foods for Human Nutrition*, 51: 79-84.
18. Akpanyung, E.O. (2005). Proximate and mineral composition of bouillon cubes

produced in Nigeria. *Pakistan Journal of Nutrition*, 4(5), 327-329. http://dx.doi.org/10.3923/pjn.2005.327.329

19. Ayoola, P.B., Adeyeye, I. and Onawumi, O.O. (2010). Trace elements and major minerals evaluation of Spondiasmombin, Vernonia amygdalina and Mmordica charantia leaves. *Pakistan Journal of Nutrition*. 9(8) : 755-756.
20. Black, R.E. (2003). Zinc deficiency, infectious disease and mortality in developing world. *Journal of Nutrition*, 133: 1485-1489.
21. Bhaskaran, P. (2011). Immunobiology of mild nutrient deficiency. *British Journal of Nutrition.*, 85: 75-80
22. Adewole, E., Ojo, A., Ogunmodede, O.T and Adewumi, D.F (2015). Antioxidant activities and nutritional compositions of *Vernonia amygdalina. International Journal of Basic and Applied Science.* 4 (1):9-16.
23. Tangka, J.K.(2003). Analysis of the thermal energy requirement for the extraction of leaf protein concentrate from some green plants. *Biosystem Engineering* 86:473-479. WHO., 1996 African traditional medicine. Afro-Tech. Rep. Ser., 1:3-4.
24. Durunna, C.S., Chiaka, I.I., Ebenebe, O.E., Udedibie, A.B., Uchegbu, M.C and Durunna, O.N (2011).Value of bitter leaf *(Vernonia amygdalina)* meal as feed ingredient in the diet of finisher. *International Journal of Agricultural Rural Development*.14:722-726.
25. Olobatoke, R.Y. and Oloniruha, J.A (2009). Haematological assessment of bitter leaf (*Vernonia amygdalina*) efficiency in reducing infections in cockerels. Proceedings of the world congress on medicinal and aromatic plants November 9-14, 2008, Cape Town, South Africa, pp 472-473.
26. Huffman, M.A., Koshimizu K.and Ohigashi, H. (1996). Ethnobotany and Zoopharmcognosy of *Vernonia amygdalina,* a medicinal plant used by humans and chimpanzees in the wild. In: Compositae: Biology and Utilization. Hind, D. J .N., P. Caligari and S.A.L . Smith (Eds.). Royal Botanical Gardens, Kew, ISBN-13: 9781900347006, pp. 351-360.
27. Adaramoye, O.A., Akintayo,O., Achem J. and Fafunso, M.A (2008). Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. *Vasc. Health Risk Management.* 4 : 235-241.
28. Mohammed, A.A and Zakariyau, A.S (2012). Bitter leaf (*Vernonia amygdalina*) as a feed additive in broiler diets. *Journal of Animal Science* 6: 38-41.
29. Chaves, A.V., Dugan, M.E.R., Stanford, K., Gibson, L.L.,Bystrom, J.M., MCAllister, T.A., Van Herk, F. and Benchaar, C.(2011). A dose-response of cinnamaldehyde supplementation on intake, ruminal fermentation, blood metabolites, growth performance and carcass characteristics of growing lambs. Livestock Sci.141:213-220.
30. Hong, J.C., Steiner, T. Aufy, A. and Lien, T.F (2012). Effects of supplemental essential oil on growth performance, lipid metabolism and immunity, intestinal characteristics, microbiota and carcass traits in broiler. Livestock Sci. 144: 253-262.
31. Goliomytis, M., Kartsonas, N., Charismiadou, M.A.,Symeon, G.K., Simitzis, P.E. and Deligeorgis, S.G of (2015). The influence naringin or hesperidin dietary supplementation on broiler meat quality and oxidative stability. PloS One. 10:e0141652.

32. Ekpo, A., Eseyin, A.O., Ikpeme, A.O and Edoho, E.J (2007). Some studies on some biochemical effects of *Vernonia amygdalina* in rats. *Asian Journal of Biochemical* 2 (3): 193-197.
33. Weber, C.and Noels, H.(2011). Atherosclerosis: Current pathogenesis and therapeutic options. Natural Medicine 17 (11): 1410-1422.
34. Owen, O.J., Amakiri , A.O. and Karibi-Botoye, T.A (2011). Lipid lowering effects of bitter leaf (Vernonia amygdalina) in broiler chickens fed finishers mash. *Agricultural Biology Journal of North America*. 2 (6): 1038-1041.
35. Nofer, J.R., Kehrel, B. and Fobver, M. (2002). HDL and arteriosclerosis: Beyond reverse cholesterol transport. Atherosclerosis, 161: 1-16.
36. Peter, M.L and Susan, C.E.F (1991). Interpretation of Laboratory results. *Australian Veterinary Practice Timer* 21 (4) pp.188-193.