MEDICINAL PLANTS LEAF MEAL SUPPLEMENTATION IN BROILER CHICKEN DIET: EFFECTS ON PERFORMANCE CHARACTERISTICS, SERUM METABOLITE AND ANTIOXIDANT STATUS

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ABSTRACT

This experiment was carried out to investigate the effect of medicinal plants leaf meal on performance characteristics, serum metabolites and antioxidant status of broiler chicken. The experiment employed a completely randomised design. All data generated were subjected to analysis of variance. A total of one hundred and forty four 4-week Abor-Acre broiler chickens were used in a finisher phase, the birds were fed with broiler finisher diets for 28 day feeding trial. The birds were assigned to 4 dietary treatments replicated three times with 12 birds per replicate. Diet I, the control diet (basal diet), Diet II contained 0.2 % bitter leaf meal (BLM), Diet III contained 0.2 % Moringa oleifera leaf meal (MOLM) and Diet IV contained 0.2 % of mixture of BLM and MOLM (1:1). The average final live-weight and daily weight gain increased (p<0.05) with the mixture of BLM and MOLM. The feed conversion ratio of birds on control diet and Diet IV were better at 1.91 and 2.03, respectively. The cholesterol levels of birds fed medicinal plant reduced significantly (p<0.05). Supplementation of diets with medicinal plants resulted in a significant (p<0.05) increase in creatinine and bilirubin. The enzyme activities test and total protein were not influenced (p>0.05) by experimental diets. The oxidative activities increased (p<0.05) in the dietary herbal supplement. It was concluded that supplementation of broiler chicken with herbs or mixture of herbs enhanced the growth performance and antioxidant capacity of birds without any deleterious effect on the health status of the birds.

Keywords: Bitter leaf meal, *Moringa oleifera* leaf meal, Serum, Anti-oxidant status

INTRODUCTION

Antibiotics have been used for more than a half of a century to improve animal performance and decreasing the pathogenic bacteria population. Unfortunately, the bacteria have established resistant strains by transferring resistance to other species between humans and animals which have resulted in serious problems in public health and livestock production (Thakare, 2004). This formed the basis for the ban of these antibiotics growth promoters by the European Union.

In recent years, extensive research has been performed on the use of phytochemicals such as bitter leaf meal and moringa leaf meal as alternative to antibiotic growth promoters in poultry diets (Mirzaei-Aghsaghali, 2012). Phytochemical screening of bitter leaf and moringa leaf meals revealed the presence of phenolic acids, flavonoids, tannins, cardiac glycosides, saponin and glucosinolates. These compounds are of great value in preventing the onset or progression of many human and animal diseases. The health promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting reactive oxygen species (Muanda *et al*., 2011).

Medicinal plants like bitter leaf meal, moringa leaf meal contain some toxins that have multi-system effects such as kidney damage (Swanepoel *et al*., 2008). Serum metabolites can also be affected by some medicinal plants; it can increase or decrease due to some damages or repairs taking place in the cells or organs (Oduola *et al.*, 2007). Ewuola and Egbunike (2008) reported that some medicinal plants are basically used as feed supplements or medicinal thereby becoming involved in a cascade of physiological reactions that may lead to alteration of serum metabolites. This could result from the toxic substances present in the plant that causes lowering or elevating the serum metabolites. It could also act as non-toxic invaluable compounds that maintain the values within the expected reference ranges for chickens (Simaraks *et al.*, 2004). This study was conducted to evaluate the role of dietary supplementation of bitter leaf meal, moringa leaf meal and the combination of bitter leaf meal and moringa leaf meal on performance characteristics, serum metabolites and antioxidant enzyme status of broiler chicken.

MATERIALS AND METHODS

Location and Duration of Feeding Trial: The feeding trial was carried out at the Poultry Unit of the Teaching and Research Farm, The Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria. The study was carried out between October to December, 2017.

Site Preparation: The poultry house was thoroughly washed and fumigated. It was allowed to stay for two weeks before the arrival of the experimental birds.

Experimental Birds: A total number of one hundred and forty four 4-week old broiler chicks of Arbo-Acre commercial breed were used in the experiment. Thirty-six birds distributed to four dietary treatments (12 birds per replicate, 36 birds per treatment) in a completely randomized design.

Preparation of Test Ingredients: The test ingredients bitter leaf meal (BLM) and *Moringa oleifera* leaf meal (MOLM) were harvested fresh from maturing stems. The fresh leaves were subjected to air-drying indoors. The air dried bitter leaf meal and moringa leaf meal were later milled to powder using a commercial feed milling machine.

Experimental Diets: The compositions of the experimental diets are presented in Tables 1.

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

Ingredients (%)	Diets			
	1	II	III	IV
	Control	Herbal supplementation		
	0.0 %	0.2 % BLM	0.2 % MOLM	0.2 % BLM + MOLM
Maize	65.00	65.00	65.00	65.00
Groundnut cake	11.00	11.00	11.00	11.00
Soybean	15.00	15.00	15.00	15.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	4.00	4.00	4.00	4.00
Oyster shell	2.00	2.00	2.00	2.00
NaCl	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Premix	0.20	0.20	0.20	0.20
Total Calculated composition	100.00	100.00	100.00	100.00
Crude protein (g/100 g)	20.31	20.31	20.31	20.31
Crude fibre (g/100 g)	2.85	2.85	2.85	2.85
M/energy (Kcal/kg)	2984.7	2984.7	2984.7	2984.7

*NaCl = Sodium chloride *Supplied per kg diet: Vit. A, 8×10^6 IU; Vit. D_3 1.2×10^6 IU; Vit. E 7×10^3 mg; Vit. 1.5×10^3 mg; Vit.B_1 2000 mg; Vit.B_2 2.5 mg; Niacin 15 g; Pantothenic acid, 5.5 g; Vit.B_6 2 g; Vit.B_{12} 10 mg; Folic acid 500 mg ; Biotin 500 mg; Choline chloride 175 g; Cobalt 200 mg; Copper 3 g; Iodine 1 g; Fe 21 g; Mn 40 g; Selenium 200 mg; Zinc 31 g*

The basal diets were formulated for the finisher phase (29 – 56 days of age) to meet NRC (1994) requirements for broilers. Thereafter, the basal diets were divided into four diets. The four diets were formulated to be iso-caloric and iso-nitrogenous. Diet I, control diet (diet without any supplementation), Diet II and Diet III contained 0.2 % of bitter leaf meal and moringa leaf meal, respectively. Diet IV contained 0.2 % mixture of bitter leaf meal and moringa leaf meal (1:1). After the broiler starter phase, birds were fed with broiler finisher diets for 28 days.

Data Collection: Data on growth performance of the experimental birds were obtained from weekly feed intake and body weight records. They were used to calculate the feed conversion ratio (ratio of feed intake to weight gain) at the end of the experiment.

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 5 ml of blood samples were collected from each bird in a two different labelled vacutainer tubes without anticoagulants and were taken to the medical laboratory for serum biochemistry test and science laboratory for antioxidant enzyme determination. The serum biochemistry tested were cholesterol, creatinine, bilirubin, aspartate transaminase, alanine transaminase and total protein. The serum samples were kept in sterile vacutainer tubes and kept deep frozen prior to analysis to determine cholesterol as outlined by Roschlau *et al*. (1974), creatinine and bilirubin were assessed by the colometric method described by Newman and Price (1999). Aspartate transaminase and alanine transaminase levels were determined as described by Huang *et al*. (2006). The total protein was determined using the method described by Peters (1968). The antioxidant enzymes such as glutathione peroxidase (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) concentrations were determined using the methods of Beutler *et al*. (1963). Enzymatic activity of SOD in the serum was determined through xanthine oxidase method. Samples were taken to detect absorbance at 550 nm with spectrophotometer. GSH activity was measured by dithio-dinitrobenzic acid method at the absorbance of 412 nm and analysed according to Beutler *et al*. (1963). The MDA level was analysed with 2-thiobarbituric acid (TBA), monitoring the change of absorbance at 532 nm with a spectrophotometer (Jensen *et al*., 1997). Proximate composition of the MOLM was determined using AOAC (2005), while flavonoid was determined using Bohm and Koupai-Abyazani (1994) method. Phytate was determined using the method of AOAC (2005), while phenol was determined by spectrophotometric method (AOAC, 2005).

Statistical Analysis: All data collected in this study were subjected to Analysis of Variance (ANOVA) using SPSS statistical package (SPSS 17.0 for widows Inc. Chicago IL, USA). Duncan's Multiple Range Test was used to separate significant mean differences. Significant differences were considered at 95 % level.

RESULTS AND DISCUSSION

The results of proximate and phytochemical compositions of bitter leaf meal (BLM) are shown in Table 2.

Table 2: Proximate analysis and phytochemicals in bitter leaf meal (BLM)

Proximate Composition (%)	
Moisture content	10.78 ± 1.58
Crude protein	15.60 ± 0.17
Crude fibre	10.58 ± 0.18
Ether extract	5.00 ± 0.10
Ash	5.57 ± 0.21
Nitrogen free extract	52.12 ± 0.47
Phytochemicals (mg/100 g)	
Flavonoid	4.84 ± 0.16
Phytate	17.53 ± 0.15
Oxalate	3.76 ± 0.05
Phenol	1.90 ± 0.17

Source: *Daramola et al. (2018a)*

The moisture content of BLM was 10.78 ± 1.05 %. The value was slightly higher than that reported 10.02 % of Asaolu *et al*. (2012). The variation may be due to soil nutrients and environmental factors which have impact on the nutrient availabilities for plants. The crude protein content (15.60 ± 1.58 %) was higher than that reported by Umit *et al*. (2011) 5.53 % for rosemary leaf. The fat content (5.00 ± 0.10 %) indicated the presence of oil in bitter leaf. The value recorded was higher than that reported by Udosen (1995). The crude fibre content was found to be 10.58 ± 0.18 %, this value fell within the range (8.50 – 20.90 %) for some vegetables from Niger Republic (Freiberger *et al*., 1998). The ash content (5.57 ± 0.21 %) was lower than that reported by

Asaolu *et al.* (2012) for bitter leaf (9.56 %). The presence of ash content in bitter leaf is a confirmation of availability of mineral elements. The nitrogen free extract content (52.12 ± 0.47 %) was higher than that reported by Asaolu *et al*. (2012) for the same plant (8.56 %) the variation may be due to soil nutrients and environmental factors which have effects on the nutrients availabilities for plants (Adewole *et al*. 2015). Phytochemicals are of benefit to health and play an active role in the the management of some diseases. The flavonoid content was 4.84 ± 0.16 mg/100g, phytate 17.53 ± 0.15 mg/100mg, oxalate was 3.76 ± 0.05 mg/100mg and phenol was 1.90 ± 0.17 mg/100g. Flavonoids have been found useful in drug preparation, in food, feed and beverages. Flavonoids from bitter leaf including phenolic acids had inhibitory activity against bacteria (Farombi and Owoeye, 2011).

The result of proximate analysis in Table 3 showed that MOLM had an appreciable crude protein (CP) content (27.95 ± 1.62 %), crude fibre (9.47 ± 0.27 %), ash (8.07 ± 1.63 %), nitrogen free extract (NFE) (46.82 ± 0.87 %) but low content of ether extract (EE) (4.96 ± 1.24).

Table 3: Proximate analysis and phytochemicals in *Moringa oleifera* leaf meal (MOLM)

Proximate Composition (%)	
Moisture content	7.42 ± 2.15
Crude protein	27.95 ± 1.62
Crude fibre	9.47 ± 0.27
Ether extract	4.96 ± 1.24
Ash	8.07 ± 1.63
Nitrogen free extract	46.82 ± 0.87
Phytochemicals (mg/100 g)	
Flavonoid	3.72 ± 0.15
Phytate	2.34 ± 0.02
Oxalate	0.65 ± 0.03
Phenol	2.74 ± 0.25

The CP value of MOLM obtained in this study was lower than the value reported by Ojo and Adetoyi (2017) which was 28.43 % although Mutayoba *et al*. (2011) recorded a higher CP (30.65 %) value. The EE and ash values 9.47 and 8.07 % observed in this study were higher than the values 2.11 and 7.93 % reported by Ogbe and Affiku (2011).

Crude fibre value of 9.47 % reported in this study was higher than 5.43 % reported in the study conducted by Sodamade *et al.* (2013). Differences in proximate values of MOLM observed in the previous studies may be due to differences in the soil type, climatic conditions, stage of maturity and the genetic make-up.

The performance characteristics of broiler chickens fed the experimental diets, indicated that the final live-weight and average daily weight gain for birds fed diets 1 and 4 were similar (p>0.05) but significantly higher (p<0.05) than birds fed diets 2 and 3 (Table 4).

The FCR of birds fed Diet II were significantly higher (p<0.05) than the FCR of birds fed other diets. The lowest FCR value was recorded for birds fed the control diet (1.91 ± 0.01), while the highest FCR was recorded for birds fed Diet II (2.41 ± 0.02). Inclusion of medicinal plant extracts in poultry diets impacted the metabolism by reducing stress and microbial activity (Sanjyal and Sapkota, 2011). The general improvement by equal mixture of BLM and MOLM on final live-weight and daily weight gain in this study revealed that the mixture of herbs (BLM and MOLM), enhanced growth performance of broiler birds. Apart from the control diets, birds fed Diet IV performed better, the combination of herbs in broiler chicken diet might have improved the production and activities of digestive enzymes. The improved performance recorded for birds on Diet IV may be attributed to the increased secretion of digestive enzyme and enhanced nutrition utilization in liver (Khan *et al*., 2012). The antibacterial action of essential component of the combination of medicinal plant may suppress the growth of pathogenic bacteria on one hand and promote the growth probiotic bacteria in the gut (Barreto *et al*., 2008). These indicated why medicinal plants may be used as alternative to antibiotic growth promoters because they exhibit antimicrobial properties and thus can form integral part of poultry nutrition (Onibi *et al*., 2009). The excellent performance of birds on diets supplemented with the mixture of those leaves suggested the

Table 4: Effect of herbal supplementation on growth performance of broiler finisher phase

Parameters	Diets			
	I	II	III	IV
	Herbal inclusion			
	0%	0.2% BLM	0.2% MOLM	0.2% BLM + MOLM
Average initial weight (g)	751.39 ± 0.02	745.90 ± 0.22	746.17 ± 0.11	749.51 ± 0.02
Average final Live-weight (g)	2045.00 ± 34.54[a]	1658.67 ± 41.93[c]	1875.00 ± 46.10[b]	2008.33 ± 44.23[a]
Average daily weight gain (g)	73.04 ± 2.00[a]	59.24 ± 1.25[c]	66.96 ± 1.56[b]	71.73 ± 1.43[a]
Total feed intake (g)	3906.84 ± 40.56[b]	3991.96 ± 48.23[b]	4237.40 ± 43.76[a]	4077.29 ± 42.54[ab]
Average daily feed intake	139.53 ± 0.42[b]	142.57 ± 0.31[b]	151.34 ± 0.25[a]	145.62 ± 0.52[ab]
Feed conversion ratio	1.91 ± 0.01[c]	2.41 ± 0.02[a]	2.26 ± 0.01[b]	2.03 ± 0.02[bc]

Means with different superscript on the same row differ significantly (p<0.05); BLM = bitter leaf meal, MOLM = Moringa oleifera leaf meal

effect of positive bioactive compounds present in both BLM and MOLM. The serum metabolites of broiler chickens fed experimental diets indicated that the serum cholesterol concentration was significantly higher (p<0.05) in birds fed control diet when compared to the treated groups. Cholesterol values of birds fed diets 3 and 4 were similar (p>0.05) but significantly higher (p<0.05) than the values recorded for birds fed Diet II (Table 5).

Table 5: Serum metabolites of broiler finisher birds fed experimental diets

Parameters	Diets			
	I	II	III	IV
	Herbal inclusion			
	0%	0.2% BLM	0.2% MOLM	0.2% BLM + MOLM
Cholesterol (mg/dl)	92.73 ± 9.18[a]	63.53 ± 10.56[c]	68.55 ± 13.24[b]	70.30 ± 11.56[b]
Creatinine (mg/dl)	0.38 ± 0.03[b]	0.98 ± 0.06[a]	0.95 ± 0.06[a]	1.18 ± 0.07[a]
Bilirubin (mg/dl)	2.17 ± 0.13[b]	2.74 ± 0.09[a]	2.88 ± 0.14[a]	2.83 ± 0.12[a]
Aspartate transaminase (IU/L)	44.17 ± 0.97[a]	40.73 ± 0.89[ab]	40.63 ± 0.84[ab]	37.51 ± 0.90[b]
Alanine transaminase (IU/L)	24.99 ± 0.12	24.93 ± 0.10	24.94 ± 0.02	25.66 ± 0.01
Total protein (g/dl)	4.10 ± 0.02	4.08 ± 0.01	4.07 ± 0.02	4.18 ± 0.03

Means with different superscript on the same row differ significantly (p<0.05); BLM = bitter leaf meal, MOLM = Moringa oleifera leaf meal

The serum concentration of creatinine and bilirubin of birds on diets 2, 3 and 4 were similar (p>0.05) but significantly higher (p<0.05) than birds fed the control diet. The aspartate transaminase concentration of birds fed the control diet was significantly higher (p<0.05) than for birds fed Diet IV. Alanine transaminase and total protein of birds were not significantly influenced (p>0.05) by the experimental diets.

Hyperlipidaemia is one of the risk factor for cardiovascular disease, while cholesterol is the major lipid constituent of atherosclerotic plaque (Daramola *et al.*, 2017). The creatinine values recorded for birds fed diets 2, 3 and 4 were within the range of chemical component in serum of chicken (0.90 – 1.85 mg/dl) (Mitruka and Rawnsley, 1977).

The low creatinine value recorded for birds fed the control diet was clinically non-significant (Saleh *et al.*, 2018). The bilirubin value recorded for birds fed the control diet was lower than the values recorded for other birds fed experimental diets. The increased levels of bilirubin could be due to an acute haemolytic disorders or lipaemia (Saleh *et al.*, 2018). The aspartate transaminase, alanine transaminase and total protein were not influenced by experimental diets.

The antioxidant properties of herbs need special attention because undesirable oxidation produces changes in odour, colour and other undesirable effects. The effect of supplementing medicinal plant on antioxidant parameters such as serum activity of glutathione (GSH), catalase (CAT) superoxide dismutase (SOD) and malondialdehyde (MDA) of broiler birds are shown in Table 6.

The GSH concentration of birds fed diets 2, 3 and 4 were similar ($p>0.05$) but significantly higher ($p<0.05$) than GSH concentration of birds fed the control diet. The catalase concentration of birds fed diets 3 and 4 were significantly higher ($p<0.05$) at 491.26 ± 5.34 μm^{-1} and 489.51 ± 7.05 μm^{-1}, respectively than catalase concentration of birds fed the control diet and Diet II at 431.48 ± 6.54 μm^{-1} and 462.94 ± 5.73 $\mu m^{-1,}$ respectively.

Table 6: Effect of herbal supplementation on antioxidant status of broiler finisher birds

Parameters	Diets			
	I	II	III	IV
	Herbal inclusion			
	0%	0.2% BLM	0.2% MOLM	0.2% BLM + MOLM
Glutathione	88.71 ± 1.37^{b}	97.33 ± 1.05^{a}	98.57 ± 1.24^{a}	99.95 ± 1.32^{a}
Catalase	431.48 ± 6.54^{c}	462.94 ± 5.73^{b}	491.26 ± 5.34^{a}	489.51 ± 7.05^{a}
SOD	94.40 ± 0.36^{c}	96.21 ± 0.39^{bc}	98.19 ± 0.44^{a}	94.85 ± 0.42^{b}
Lipid peroxidation	13.56 ± 0.47^{a}	10.14 ± 0.52^{b}	9.71 ± 0.45^{b}	8.71 ± 0.54^{c}

Means with different superscript on the same row differ significantly (P<0.05); BLM = bitter leaf meal, MOLM = Moringa oleifera leaf meal, SOD = Superoxide dismutase

Serum SOD activity was significantly increased ($p<0.05$) in birds fed Diet III and reached a maximum (98.19 ± 0.44 μm^{-1}) with MOLM supplemented diet, while the least activity was recorded for birds fed control diet (94.40 ± 0.36 μm^{-1}). The lipid peroxidation concentration of birds fed control diet was significantly higher ($p<0.05$) than lipid peroxidation concentration of birds fed diets 2, 3 and 4 (10.14 ± 0.52 nmol/ml, 9.7 ± 0.45 nmol/ml and 8.74 ± 0.54 nmol/ml) respectively.

These results conformed with the report of Daramola *et al.* (2018 a, b) that the intake of herbs or medicinal plants or their contents resulted in increased serum antioxidant enzyme such as GSH, catalase and SOD and a decreased lipid peroxidation concentration. Elevated levels of antioxidant enzymes may improve the steady state of the system of broiler birds. The results of this study demonstrated that phytochemicals such as flavonoid, quercetin and phenol were identified as the most potent antioxidants in moringa leaves and bitter leaf (Atawodi *et al.*, 2010). Adewole *et al.* (2015) reported that BLM and MOLM possess some antioxidative properties with high nutritive values; the leaves are rich in mineral and other essential phytochemicals. The antioxidants may be used as defence system to prevent free radicals from damaging the cells and organs of the body, and against infections and degenerative diseases (Sreelatha and Padma, 2009). An over production of reactive system can cause the imbalance of defense system therefore, antioxidants are needed which focus on natural compounds from natural sources. From this study, supplementation with mixture of BLM and MOLM increased the activities of GSH and catalase but decreased the MDA concentration in the serum of broiler chickens. The higher the activities of GSH, catalase in diets 3 and 4 the higher the capacity of broiler chicken to clear out the oxygen free radicals i.e. reactive oxygen species (ROS) and lower MDA concentration. The increase in the GSH, catalase and SOD may be due to the enhanced antioxidant status by MOLM and mixture of BLM and MOLM which is likely attributed to the antioxidant compounds.

Conclusion: It can therefore be concluded that supplementation of broiler finisher birds with herbs or the mixture of herbs enhanced the antioxidant capacity of broiler chicken without any deleterious effect on the serum biochemistry of the experimental birds. Further studies can be done to determine the use of BLM and MOLM in broiler chickens.

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REFERENCES

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.

AOAC (2005). *Official Methods of Analysis*. 16th Edition, Association of Official Analytical Chemists (AOAC), Washington DC, USA.

ASAOLU, S. S., ADEFEMI, O. S., OYAKILOME, I. G., AJIBULU, K. E. and ASAOLU M. F. (2012). Proximate and mineral composition of Nigeria leafy vegetable. *Journal of Food Research*, 1(3): 214 – 218.

ATAWODI, S., ATAWODI, J., IDAKUN, G., PFUNDSTEIN, B., HAUBNER, R., WURTELE, G., BARTSCH, H. and OWEN, R. (2010). Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of *Moringa oleifera* Lam. *Journal of Medicinal Food*, 13(3): 710 – 716.

BARRETO, M. S. R., MENTEN, J. F. M., RACANICCI, A. M. C., PEREIRA, P. W. Z. and RIZZO, P. V. (2008). Plant extracts used as growth promoters in broilers. *Brazillian Journal Poultry Science*, 10(2): 109 – 115.

BEUTLER, E., DURON, O. and KELLY M. (1963). Improved method for the determination of blood glutathione. *Journal of Laboratory Clinical Medicine,* 61: 882 – 888.

BOHM, B. A. and KOUPAI-ABYAZANI, M. R. (1994). Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium reticulatum* and *V. calycinum* (Ericaceae). *Pacific Science,* 48(4): 458 – 463.

DARAMOLA, O. T, OLORUNTOLA, O. D. and AYODELE, S. O. (2018a). Nutritional evaluation of bitter leaf meal (*Vernonia amygdalina*): effects on performance, carcass and serum metabolites of broiler chickens. *Nigerian Journal of Animal Science,* 20(2): 211 – 221.

DARAMOLA, O. T., JIMOH, A. O., OLORUNTOLA, O. D. and AYODELE, S. O. (2018b). Effect of herbal supplement on the laying performance and antioxidant status of serum and egg yolk of laying birds. *Biotechnology Journal International,* 21(3): 1 – 9.

DARAMOLA, O. T., OLORUNTOLA, O. D. and AYODELE, S. O. (2017). Effect of varying levels of *Vernonia amygdalina* leaf meal on lipid profile and serum metabolites of broiler chickens. Pages 623 – 626. *In*: ADELOYE, A. A., OYAWOYE, E. O., TOYE, A. A. and ADESINA, B. T. (Eds.). *Proceeding of the 42nd Annual Conference, Nigerian Society for Animal Production (NSAP).* Held at Landmark University, Omu-Aran, Kwara State, Nigeria, 26 – 30th March, 2017.

EWUOLA, E. O. and EGBUNIKE, G. N. (2008). Haematological and serum biochemical growing rabbit bucks fed dietary fumonisin B_1. *African Journal of Biotechnology,* 7(23): 4304 – 4309.

FAROMBI, E. O. and OWOEYE, O. (2011). Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. *International Journal of Environmental Research and Public Health*, 8(6): 2533 – 2555.

FREIBRGER, C. E., VANDERJAGT, D. J., PASTUSZYN, A., GLEW, R. S., MOUNKAILA, G., MILLSON, M. and GLEW, R. H. (1998). Nutrient content of the edible leaves of seven wild plants from Niger. *Plant Foods for Human Nutrition,* 53(1): 57 – 69.

HUANG, X. J., CHOI, Y. K., IM, H. S., YARIMAGA, O., YOON, E. and KIM, H. S. (2006). Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. *Sensors*, 6(7): 756 – 782.

JENSEN, C., ENGBERG, R., JAKOBSEN, K., SKIBSTED, L. H. and BERTELSEN, G. (1997). Influence of the oxidative quality of dietary oil on broiler meat storage stability. *Meat Science*, 47(3-4): 211 – 222.

KHAN, R. U., NAZ, S., NIKOUSEFAT, Z., TUFARELLI, V., JAVDANI, M., QURESHI, M. S. and LAUDADIO, V. (2012). Potential applications of ginger (*Zingiber officinale*) in poultry diets. *World's Poultry Science Journal*, 68(2): 245 – 252.

MIRZAEI-AGHSAGHALI, A. (2012). Importance of medical herbs in animal feeding: a review. *Annals of Biological Research*, 3(2): 918 - 923.

MITRUKA, B. M. and RAWNSLEY, H. M. (1977). *Clinical, Biochemical and Haematological Reference Values in Normal Experimental Animals.* Masson Publishing USA Incorporated, New York, USA.

MUANDA, F., KONE, D., DICKO, A., SOULIMANI, R. and YOUNOS, C. (2011). Phytochemical composition and antioxidant capacity of three Malian medicinal plant parts. *Evidence-Based Complementary and Alternative Medicine,* 2011: http://dx.doi.org/10.1093/ecam/nep109.

MUTAYOBA, S. K., DIERENFELD, E., MERCEDES, V. A., FRANCES, Y. and KNIGHT, C. R. (2011). Determination of chemical composition and ant-nutritive components for Tanzanian locally available poultry feed ingredients. *International Journal of Poultry Science,* 10(5): 350 – 357.

NEWMAN, D. J. and PRICE, C. P. (1999). Renal function and nitrogen metabolites. Pages 1204 – 1270. *In*: BURTIS, C. A. and ASHWOOD, E. R. (Eds.). *Tietz Textbook of Clinical Chemistry*. 3rd Edition, W. B. Saunders Company, Philadelphia.

NRC (1994). *Nutrient Requirement of Poultry*. 9th Revised Edition, National Research Council (NRC), National Academy Press, Washington DC, USA.

ODUOLA, T., POPOOLA, G. B., AVWORO, O. G., ODUOLA, T. A., ADEMOSUN, A. A. and LAWAL, M. O. (2007). Use of *Jatropha gossypifolia* stem latex as a haemostatic agent: how safe is it? *Journal of Medicinal Plants Research*, 1(1): 14 – 17.

OGBE, A. O. and AFFIKU, J. P. (2011). Proximate study, mineral and anti-nutrient composition of *Moringa oleifera* leaves harvested from Lafia, Nigeria: potential benefits in poultry nutrition and health. *Journal of Microbiology, Biotechnology and Food Sciences*, 1(3): 296 – 308.

OJO, O. A. and ADETOYI, S. A. (2017). Effect of *Moringa oleifera* leaf extract on the haematological and serum biochemistry of rabbits reared in a semi-humid environment. *African Journal of Biotechnology,* 16(25): 1386 – 1390.

ONIBI, G. E., ADEBISI, O. E. and FAJEMISI, A. N. (2009). Response of broiler chicken in terms of performance and meat quality of garlic (*Allium sativum*) supplementation. *African Journal of Agricultural Research*, 4(5): 511 – 517.

PETERS, T. (1968). Proposals for standardization of total protein assays. *Clinical Chemistry*, 14(12): 1147 – 1159.

ROSCHLAU, P., BERNT, E. and GRUBER, W. (1974). Enzymatic determination of total cholesterol in serum. *Zeitschrift fur klinische Chemie und klinische Biochemie,* 12(9): 403 – 407.

SALEH, A. A., ALI, H., ABDEL-LATIF, M. A., EMAM, M. A., GHANEM, R. and EL-HAMID, H. S. A. (2018). Exogenous dietary enzyme formulations improve growth performance of broiler chickens fed a low-energy diet targeting the intestinal nutrient transporter genes. *PloS One,* 13(5): e0198085. https://doi.org/10.1371/journal.pone.0198085

SANJYAL, S. and SAPKOTA, S. (2011). Supplementation of broilers diet with different sources of growth promoters. *Nepal Journal of Science and Technology*, 12: 41 – 50.

SREELATHA, S. and PADMA, P. R. (2009). Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods for Human Nutrition*, 64(4): 303.

SIMARAKS, S., CHINRASRI, O. and AENGWANICH, W. (2004). Hematological, electrolyte and serum biochemical values of the Thai indigenous chickens (*Gallus domesticus*) in northeastern, Thailand. *Songklanakarin Journal of Science and Technology*, 26(3): 425 – 430.

SODAMADE, A., BOLAJI, O. S. and ADEBOYE, O. O. (2013). Proximate analysis, mineral contents and functional properties of *Moringa oleifera* leaf protein concentrate. *IOSR Journal of Applied Chemistry*, 4(6): 47 – 51.

SWANEPOEL, C., BLOCKMAN, M. and TALMUD, J. (2008). Nephrotoxins in Africa. Pages 859 – 870. *In*: DE BROE, M. E., GEORGE, A. P., BENNETT, W. M. and DERAY, G. (Eds.). *Clinical Nephrotoxins: Renal Injury from Drugs and Chemicals*. 3rd Edition, Springer Science, New York, NY 10013, USA.

THAKARE, M. (2004). Pharmacological screening of some medicinal plants as antimicrobial and feed additives. MSc. Dissertation, Virginia Polytechnic, Institute and State University. Virginia, USA.

UDOSEN, E. O. (1995). Proximate and mineral composition of some Nigerian vegetables. *Discovery and Innovation,* 7(4): 383 – 386.

UMIT, P., DERYA, Y. and MUSTAFA, E. (2011). Serum biochemical profile of broiler chickens fed diets containing rosemary and rosemary volatile oil. *Journal of Biology and Environmental Science,* 5(13): 23 – 30.