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Effect of Bitterleaf (*Vernonia amygdalina*) meal on Haematology and Serum Chemistry of Broiler chickens

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Abstract

Feeding is one of major components that determines performance of Broiler chickens. However, high cost of Broiler chicken production in the recent times has been equally attributed to extra cost incurred from the importation of some feed additives that enhance performance of the birds. Therefore, exploring locally available plant materials that have been proven to posses antibiotics properties could be considered important in order to reduce cost of feeding due to importation of antibiotics. This study was conducted to evaluate effect of Bitterleaf (Vernonia amygdalina) meal in Broiler chicken diets on haematology and serum chemistry. Four experimental diets T_1 (control, 0% BLM), T_2 (5% BLM), T_3 (10% BLM) and T_4 (15% BLM) were fed to 120 Broiler chickens replicated three times in a Completely randomized design. Birds were offered feed and water ad-libitum. Data were collected on haematology and serum chemistry parameters. T_3 was significantly ($P<0.05$) higher than others on values of PCV and RBC. No significant ($P>0.05$) difference were recorded in Hb and WBC of T_2 and T_3. Meanwhile, T_4 was ($P<0.05$) higher than T_2 and T_3 in WBC and T_2 was ($P<0.05$) higher than that of T_3 and T_4 in MCH and MCHC. T_2 and T_3 were ($P<0.05$) higher than T_4 in lymphocytes, while T_3 and T_4 were ($P<0.05$) higher than T_2 in the monocytes. For serum chemistry, T_3 was ($P<0.05$) higher than T_2 and T_4 in total protein and albumin. There was ($P<0.05$) difference in the cholesterol content of the birds. Haematology and serum chemistry values recorded were within the standard ranges for good health condition of the Broiler chickens. Birds fed 5% Bitterleaf meal performed better than others. Therefore, 5% Bitterleaf meal in the diets of Broiler chickens producing birds with high immunity.

Key words: *Vernonia amygdalina* meal, haematology, serum chemistry, Broiler chicken

Effet de la farine de feuilles amères (Vernonia amygdalina) sur l'hématologie et la chimie sérique des poulets de chair

Résumé

L'alimentation est l'un des principaux composants qui déterminent les performances des poulets de chair. Cependant, le coût élevé de la production de poulets à griller ces derniers temps a également été attribué aux coûts supplémentaires occasionnés par l'importation de certains additifs alimentaires qui améliorent les performances des oiseaux. Par conséquent, l'exploration de matières végétales disponibles localement et dont il a été prouvé qu'elles possèdent des propriétés antibiotiques pourrait être considérée comme importante afin de réduire le coût de l'alimentation en raison de l'importation d'antibiotiques. Cette étude a été menée pour évaluer l'effet de la farine de Bitterleaf (Vernonia amygdalina) dans l'alimentation des poulets de chair sur l'hématologie et la chimie du sérum. Quatre régimes expérimentaux i. e T1 (témoin, 0 % BLM), T2 (5 % BLM), T3 (10 % BLM) et T4 (15 % BLM)

ont été donnés à 120 poulets de chair répliqués trois fois dans un modèle entièrement randomisé. Les oiseaux ont reçu de la nourriture et de l'eau à volonté. Des données ont été recueillies sur les paramètres d'hématologie et de chimie sérique. T3 était significativement (P < 0,05) plus élevé que les autres sur les valeurs de PCV et RBC. Aucune différence significative (P> 0,05) enregistrée dans l'Hb et le WBC de T2 et T3. Pendant ce temps, T4 était significativement (P <0, 05) supérieur à T2 et T3 dans WBC et T2 était significativement (P <0, 05) supérieur à celui de T3 et T4 dans MCH et MCHC. T2 et T3 étaient significativement (P<0,05) supérieurs à T4 dans les lymphocytes, tandis que T3 et T4 étaient significativement (P<0,05) supérieurs à T2 dans la quantité de monocytes. Pour la chimie du sérum, T3 était significativement (P < 0,05) supérieur à T2 et T4 en protéines totales et en albumine. Il y avait une différence significative (P<0,05) dans la teneur en cholestérol des oiseaux. Les valeurs d'hématologie et de chimie sérique enregistrées se situaient dans les plages standard pour un bon état de santé des poulets de chair. Les oiseaux nourris avec 5 % de farine de Bitterleaf ont obtenu de meilleurs résultats que les autres. Par conséquent, 5% de farine de Bitterleaf dans l'alimentation des poulets à griller produisant des oiseaux à haute immunité.

Mots-clés : Tourteau de Vernonia amygdalina, hématologie, chimie sérique, Poulet de chair

Introduction

Poultry farming has emerged as one of the fastest growing agribusiness enterprises in the world. Expansion of this enterprise would help in feeding the expanding population with adequate animal protein. Among other factors that greatly affect poultry production in the tropics, feeding constitutes a major constraint due to high cost of feed ingredients, especially those that are not locally available.

The use of medicinal plants in livestock and poultry feed /diet have shown great improvement in growth performance. Although, they possess anti-nutritional factors, which can be eliminated or reduced drastically through the use of different processing methods. They have shown far better results compared to synthetic feed additives. One of the major feed additives that can be used is *Vernonia amygdalina* (Bitterleaf) meal.

Bitterleaf (*Vernonia amygdalina*) is a perennial shrub from Asteraceae family and one of the edible vegetables that grow throughout the tropical Africa. It is popularly called Bitterleaf because of its abundant bitter taste (Ekpo *et al.,* 2007). It performs both medicinal and nutritive functions (Fasuyi, 2006; Adaramoye *et al*., 2008; Oseni and Babatunde, 2016). The leaf of *Vernonia amygdalina* contains a considerable amount of anti-nutritional factors like high level of tannic acid and saponin (Charles and Boulevard, 2012). Akwaowo *et al*., (2010) reported that the young leaves often preferred for human consumption, contains high cyanide ($60.10mg100^{-1}g$ DM), and tannin content ($40.6mg100^{-1}g$ DM) than the older leaves. Research has shown that *Vernonia amygdalina* (either the leaf meal or the aqueous extract) has some beneficial effects in disease management of poultry such as anti-coccidiosis, anti-bacterial and anti-parasitic (Dakpogan, 2006; Akinyele *et al*., 2014; Gbolade, 2009; Tadesse *et al*., 1993), as an anti-oxidant (Erasto *et al*., 2007) and as a growth promoter by enhancing the gastro intestinal enzymes thus increasing feed conversion efficiency (Huffman *et al*., 1996; Olobatoke and Oloniruha, 2009).

Several studies carried out on this plant had suggested that it contains different bioactive compounds, including, flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides,

triterpenoids, and several types of sesquiterpene lactones (Farombi and Owoeye, 2011; Adedapo *et al.*, 2014). These bioactive compounds made them possess different pharmacological properties like antimicrobial, antioxidant, laxative, hypoglycemic, antihelmintic, anti-inflammatory, among others (Igile *et al.*, 1994; Akinpelu, 1999 Erasto *et al.*, 2007; Yeap *et al*., 2010; Farombi and Owoeye, 2011; Anibijuwon *et al.*, 2012).

On the strenght of the facts mentioned above, the study was carried out to evaluate effect of Bitterleaf meal (*Vernonia amygdalina*) in Broiler chicken diets on haematology and serum chemistry in Umudike, Abia state of Nigeria.

Materials and methods

Experimental site

The study was carried out at the Poultry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria (MOUAU). The area falls within the Tropical rain forest zone, it is located at latitude 05° 21°N and longitude 07° 33°E, its elevation is about 112m above sea level. It has an average Rainfall of about 2177mm/annum, Relative Humidity of about 50-90% and a monthly temperature range of 17°-36°C (Meteorological Station, NRCRI, Umudike, 2018).

Experimental birds and management

One hundred and twenty day old Ross (308). Broiler chicks were purchased and brooded for one week for stabilization using kerosene lamps, stove and electric bulbs as sources of heat. The birds were fed straight diets *ad libitum.* The birds were allotted to four dietary treatments at 30 birds per treatment and replicated thrice at 10 birds per replicate) during the seven weeks that the experiment lasted. All the vaccination and other routine poultry management practices were observed.

Experimental materials

Fresh bitter leaves were purchased from Ubani market at Umuahia and environs. It was dried at room temperature for seven days before it was milled using hammer mill. The milled bitter leaves were subjected to proximate composition Analysis.

Data collection

Data was collected at the end of the experiment. A bird from each replicate whose life weight was closest to the mean weight of the birds was randomly selected and blood samples were collected from the right jugular vein into sample bottles containing Ethylene Diamond Tetra Acetic Acid (EDTA) for hematological determinations. The sample bottle was gently shaken to mix up the blood with (EDTA) to prevent clotting. Blood samples for biochemistry determinations were collected into clean sample bottles without anticoagulant. Serum was obtained by allowing the blood samples to clot at room temperature for 30 minutes, after which they were centrifuged for 10 minutes at 3,000 revolutions per minute using a table centrifuge to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was carefully aspirated and stored.

The packed cell volume (PCV) was determined by the micro-hematocrit Method. The haemoglobin concentration (Hb) of the blood samples was determined by the cyano- methaemoglobin method. Red blood cell (RBC) and total white blood cells determined by the haemo-cytometer method as outlined by Akintunde *et al.* (2017).

Biochemical parameters

Total protein was determined by direct Burette method for *in-vitro* determination of total protein in serum as plasma. The Serum cholesterol was determine by enzymatic colorimetric method (Mitruka and Rawnskey, 1997) for the *in- vitro*

determination of cholesterol in Serum using a QCA enzymatic cholesterol test kit (Quimica Clinica Aplicada, Spain). The Serum Urea was determined by the modified Berthelot- Secrecy method for the *in-vitro* determination of Urea in Serum (Mitruka and Rawnskey, 1997), using QCA enzymatic Urea test kit. The blood glucose level (mg/dl) was determined using the Accu-check active diabetes monitoring kit based on the glucose oxide method by Roche diagnostic.

Experimental design and statistical analysis

In this experiment, Completely Randomized Design (CRD) was used, and all data collected was subjected to analysis of variance (ANOVA) as outlined by Steel and Torrie (1980). The mean separation was done using Duncan's new multiple range test (Duncan,1955) where significance occurred.

Results and discussion

The result of the hematological indices of broiler chickens feed varying levels of bitter leaf meal is presented in Table 2. All the parameters investigated showed significant ($P<0.05$) differences among the means. Haemoglobin were observed to be 12.13g/dL, 11.70g/dL, 11.62g/dL and 11.14g/dL for T_1, T_2, T_3 and T_4 respectively. Red blood cells were 3.61 x 10^6mm^3, 3.29 x10^6mm^3, 3.57 x 10^6mm^3 and 3.46 x 10^6mm^3 for T_1, T_2, T_3 and T_4 respectively while, white blood corpuscles recorded were 20.23 x10^6mm^3, 23.30 x 10^6mm^3, 24.10 x 10^6mm^3 and 25.50 x 10^6mm^3 for T_1, T_2, T_3 and T_4 respectively. Birds fed control diet had PCV value of 31.67% while those fed diets containing 5%, 10% and 15% *Vernonia amygdalina* leaf meal recorded PCV values of 29.33%, 31.33% and 29.67% respectively. 5% *V.amygdalina* had the highest values of MCH and MCHC. 10% and 15% *V.amygdalina* recorded MCH values of 32.54pg and 32.23pg respectively and MCHC of 37.07% and 37.57% respectively.

The haemoglobin values obtained in this study ranged from 11.14 – 12.13g/dL which were within the normal range for broiler chickens of 7.0 – 13.0g/dL as reported by Nwaoguikpe (2010). The highest value obtained in this study was observed in birds of treatment 1 (control diet) while the least value was of birds of treatment 3 (10% inclusion level) which was not significantly ($p>0.05$) different from birds fed diet 2 (5% inclusion level). The Hb value observed in the diets containing *V.amygdalina* compared with the control diet, suggesting that bitter leaf meal may possess high quality protein and iron thus when added to their feed supplied sufficient blood pigment to the animal for adequate transportation of oxygen around the body. This is confirmed by the red blood corpuscles which were not significantly ($P<0.05$) different from one another. RBC specializes in the transportation of oxygen and carbon dioxide to and fro the body tissues as well as production of the haemoglobin. The values of RBC obtained in this study were still within the normal range of 2.0 – 4.0 x10^6mm^3 for broiler chicken (Mitruka and Rawnskey, 1997; Akinola and Abiola, 1991)

WBC is one of the most important hematology assays in the assessment of health status of animal. The range values obtained for WBC ranged from 20.23 x 10^6mm^3 - 25.50 x 10^6mm^3. Significant differences ($p<0.05$) exists among the treatment groups. Diets containing *V. amygdalina* were observed to have higher ($P<0.05$) values of white blood corpuscles than the control diet. There were no significant ($P>0.05$) difference between treatments 2 and 3. However the WBC values obtained from this study were still within the normal range of a healthy chicken of 9 – 31 x10^3mm^3 Mitruka and

Rawnskey (1997).

The PCV did not show any particular trend but they were observed to be within the normal range of 22% -35% as reported by Mitruka and Rawnskey (1997) suggesting that *V. amygdalina* does not have any adverse effect on PCV values of broiler birds. This is evidenced in the Hb content which were within normal range for broiler chickens.

The MCV obtained in this study ranged between 85.83fl and 89.16 fl and were lower range of birds of 90 – 140fl observed by Ijeh and Ejike (2011). Mean corpuscular volume (MCV) expresses the average volume of individual red blood cells. It aids in determining the presence of anaemia that affect an animal. Low MCV may indicate iron deficiency, chronic disease, hemoglobin disorder such as anaemia due to blood cell destruction or bone marrow disorders as reported by Akah *et al*. (2009).

For MCH, there were no significant ($p>0.05$) differences between treatments 3 and 4 which in turn was significantly ($p<0.05$) different from the rest of the treatment groups. The values obtained ranged from 32.23pg - 35.56 pg. and were within the normal range of MCH of 32.0 – 43.9 pg reported by Ross *et al.* (1978) and 33.0 – 47.8 pg reported by Ijeh and Ejike (2011).

The MCHC values in all the treatments ranged from 37.07% -39.80% and were within the normal range of 30 – 47 g/dl reported by Mmereole (1996). There were no significant ($p>0.05$) differences among treatments 1, 3 and 4 but significantly ($p<0.05$) different from treatment 2.

White blood differentials of Broiler chickens fed varying levels of Bitterleaf meal

The result of white blood differentials of broiler chicken fed varying levels of bitter leaf meal is presented in Table 4. Neutrophils and eosinophil which did not show any significant differences ($p>0.05$), while, significant differences ($P<0.05$) in mean existed among the lymphocytes and monocytes. Neutrophils values obtained ranged from 19.00% – 21.33%. Neutrophil which is part of white blood cell is one of the most important haematological parameters for the assessment of health status of a healthy bird, therefore higher values shows healthier birds. The result obtained in this study showed that bitter leaf meal has nutrients and capacity to fight against invading diseases and improve the health status of the birds.

Lymphocytes showed no significant differences ($p>0.05$) for birds fed dietary treatments 1, 2, and 3 but significantly ($p<0.05$) different from treatment 4 birds. The values ranged from 66.67% -70.67%. However these values were higher than the range of 50 – 62% for healthy birds as opined by Imaga and Bamigbetan (2013).

Serum biochemistry of Broiler chickens fed varying levels of Bitterleaf meal

The result of serum biochemistry of broiler chickens fed varying levels of bitterleaf meal is presented in Table 4. There were significant ($p<0.05$) differences among all the parameters measured such as total protein, albumin, globulin, glucose, cholesterol and urea.

There were no significant ($P>0.05$) differences in the total protein values of birds fed diets 1, 3 and 4 while the birds fed diet 2 ($P<0.05$) differed from the others. The values obtained ranged from 2.83g/dL - 3.54g/dL which, were within the range values of normal birds of 3.25g/dL – 7.61 g/dL (Ross *et al*., 1978) and 3.3g/dL – 5.5 g/dL reported by Oguwike *et al.* (2013). Nevertheless, birds fed dietary treatment 2 were below the range. Low total protein level could result to malnutrition, disease condition causing protein loss, liver failure and renal loss.

The Albumin values obtained were between 1.34g/dL - 1.79 g/dL. Except for treatment 3, other treatment groups did not show any

Table 1: Proximate composition of Bitterleaf (*V. amygdalina*) meal and the experimental diets

	Bitterleaf meal	T1 (0% BLM)	T2 (5% BLM)	T3 (10% BLM)	T4 (15% BLM)
DM (%)	84.51	91.84	91.20	91.14	91.08
MC (%)	15.49	8.16	8.80	8.86	8.92
Ash (%)	15.02	7.28	8.04	8.43	8.87
CP (%)	17.75	20.85	19.53	18.25	17.75
EE (%)	0.30	5.62	5.55	5.55	5.50
CF (%)	1.65	4.46	4.49	4.56	4.72
NFE (%)	49.79	53.63	53.59	54.35	54.24
ME (Kcal/kg)	2219.73	2903.41	2848.49	2824.84	2804.40

DM, MC, CP, EE, CF, NFE and ME represent dry matter, moisture content, crude protein, ether extract, crude fibre, Nitrogen-free extract and metabolizable energy

Table 2: Haematological indices of Broiler chickens fed varying levels of Bitterleaf meal

Parameters	T1 (0% BLM)	T2 (5% BLM)	T3 (10% BLM)	T4 (15% BLM)	SEM
Hb (g/dl)	12.13^{a}	11.70^{b}	11.62^{b}	11.14^{c}	0.11
RBC ($x10^{6}mm^{3}$)	3.61^{a}	3.29^{c}	3.57^{a}	3.46^{b}	0.39
WBC ($x10^{6}mm^{3}$)	20.23^{c}	23.30^{b}	24.10^{b}	25.50^{a}	0.61
PCV (%)	31.67^{a}	29.33^{b}	31.33^{a}	29.67^{b}	0.34
MCV (fl)	87.72^{ab}	89.16^{a}	87.79^{ab}	85.83^{b}	0.52
MCH (pg)	33.60^{b}	35.56^{a}	32.54^{c}	32.23^{c}	0.40
MCHC (%)	38.31^{b}	39.89^{a}	37.07^{b}	37.57^{b}	0.36

$^{a-b-c}$:means along the same row with different superscripts are significantly (<0.05) different. SEM= Standard error of mean. BLM= Bitterleaf meal, Hb=Haemoglobin, RBC=Red blood cell, PCV=Packed cell volume, MCV=Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration.

Table 3: White blood differentials of Broiler chickens fed varying levels of Bitterleaf meal

Parameters	T1 (0% BLM)	T2 (5% BLM)	T3 (10% BLM)	T4 (15% BLM)	SEM
Neutrophils (%)	20.00	20.00	19.00	21.33	0.51
Lymphocytes (%)	70.67^{a}	69.00^{a}	69.33^{a}	66.67^{b}	0.53
Eosinophils (%)	3.67	4.00	4.00	4.00	0.19
Monocytes (%)	5.67^{b}	7.00^{b}	7.67^{a}	8.00^{a}	0.33

$^{a-b}$:means along the same row with different superscripts are significantly (<0.05) different. SEM= Standard error of mean.
BLM= Bitterleaf meal

Table 4: Serum biochemistry of Broiler Chickens fed varying levels of Bitterleaf meal

Parameters	T1 (0% BLM)	T2 (5% BLM)	T3 (10% BLM)	T4 (15% BLM)	SEM
Total protein (g/dl)	2.99^{ab}	2.83^{b}	3.54^{a}	3.08^{ab}	0.11
Albumin (g/dl)	1.47^{b}	1.34^{b}	1.79^{a}	1.51^{b}	0.06
Globulin (g/dl)	1.51	1.49	1.75	1.58	0.06
Glucose (mg/dl)	258.33^{a}	240.00^{b}	229.33^{b}	211.33^{c}	5.35
Cholesterol (mg/dl)	176.19^{a}	149.38^{b}	121.40^{c}	125.17^{c}	6.94
Urea (mg/dl)	4.43	4.55	4.62	4.78	0.07

$^{a,b-c}$:means along the same row with different superscripts are significantly ($p < 0.05$) different. SEM= Standard error of mean, BLM= Bitterleaf meal.

significant differences (p>0.05). However, the values obtained were within the reference values of normal birds ranging from 1.3 – 2.8 g/dL.

Globulin values obtained in this study ranged from 1.49g/dL - 1.75g/dL. None significant (P>0.05) differences existed among the treatment groups and values obtained were within the range of a healthy bird 1.5 – 4.1 g/dL (Osinubi, 2008). Treatments 2 and 3 did not show any significant differences (p>0.05) in glucose level, but significantly different (p<0.05) from the rest of treatment groups. The values obtained ranged between 211.33mg/dL - 258.33mg/dL. Birds fed 15% *V. amgdalina* leaf meal had the lowest glucose level while the highest glucose was observed in birds fed 0% *V. amygdalina.* The serum glucose appeared to decrease as the level of *V. amygdalina* increased in the diets. However, the glucose level in the blood of all the birds fed the different diets were higher than the normal range of a healthy bird of 126 – 204 mg (Owolabi, *et al.,* 2008). Increased level of glucose indicates hypergylycemia implicating diabetes (Osinubi, 2008). The range values obtained were 121.40mg/dL - 176.19mg/dL. Urea obtained ranged from 4.43mg/dl to 4.78mg/dl and there no significant differences (p>0.05) among the treatment groups. The urea values were within the normal range of 2.5mg/dL – 8.1 mg/dL for a healthy bird according to Imaga and Bamigbetan (2013). The high level of urea value may be an indication of poor feed quality.

Conclusion

The performance of Broiler chickens fed diets containing varying levels of *Vernonia amygdalina* with respect to haematological indices and serum chemistry and white blood differentials, indicated non adverse effect of the *Vernonia amygdalina* up to 15% inclusion level. This suggests that Bitterleaf meal (*Vernonia amygdalina*) conveyed a nutritional benefit, which in turn improved the health of the Broiler chickens and could therefore be used as alternative to antibiotics, thus supporting healthy birds and minimizing the cost of production.

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