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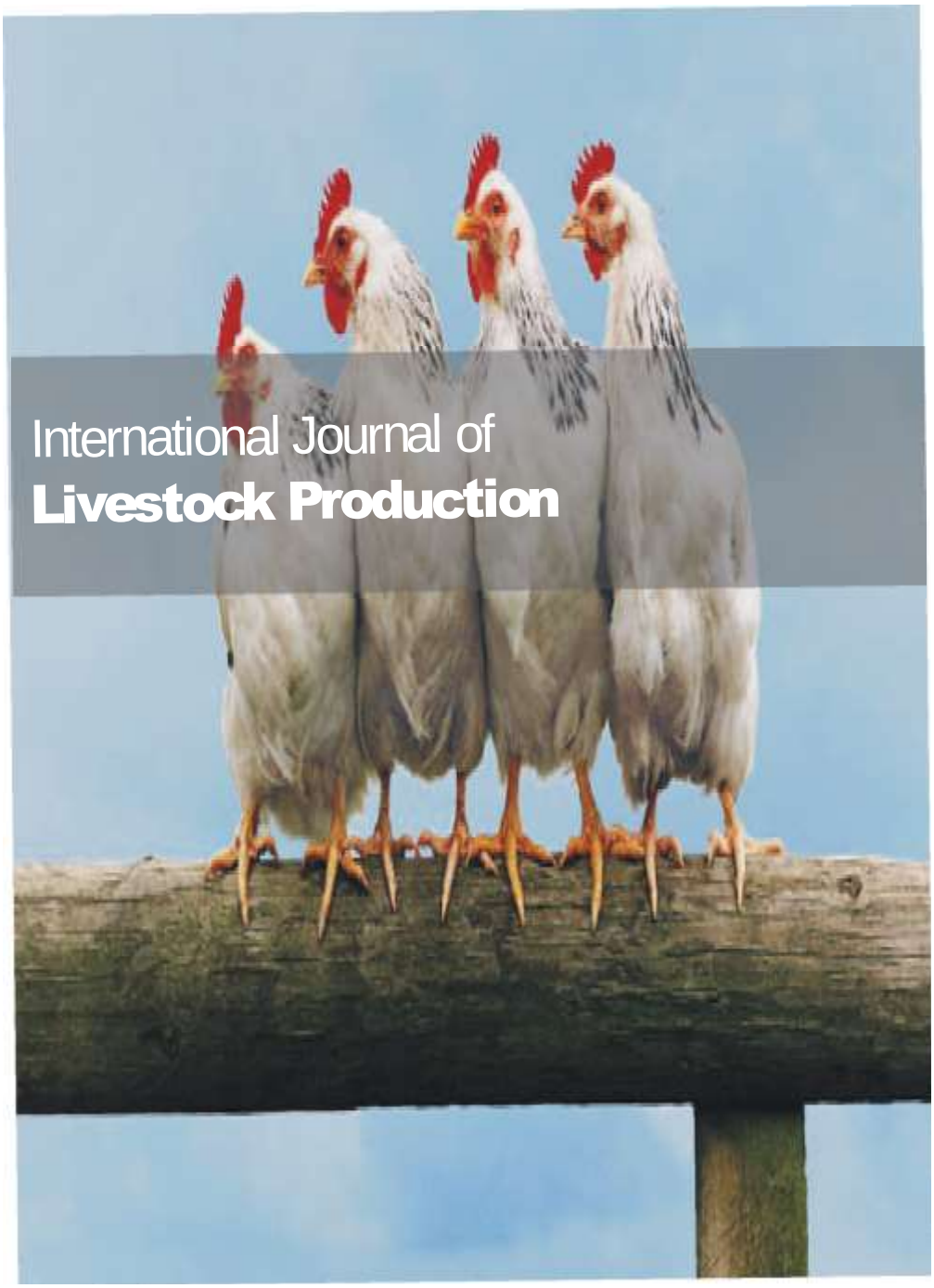

International Journal of Livestock Production

August 2019
ISSN 2141-2448
DOI: 10.5897/IJLP
www.academicjournals.org

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Vol. 10(8), pp. 188-191, August 2019
DOI: 10.5897/IJLP2018.0512
Article Number: 728352961603
ISSN 2141-2448

http://www.academicjournals.org/IJLP

ACADEMIC JOURNALS

International Journal of Livestock Production

Full Length Research Paper

Evaluation of proximate content and vitamin profile of *Moringa oleifera* seed hull

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Received 28 July, 2018; Accepted 19 October, 2018

Moringa plant is a plant with increase awareness of its health and nutritional benefits in Nigeria. This study was conducted to examine the nutrient composition of underutilized hull of *Moringa oleifera* seed. Proximate composition, antinutrients, minerals and vitamins profile of the hull were determined using standard analytical methods. The hull contained crude protein 5.25±0.011%, crude fibre 19.5±0.038%, ash 5.0±0.026%, crude lipid 4.25±0.011%, carbohydrate 55.2±0.062 and moisture content of 10.8±0.033%. Sodium (45.49 mg/100 g) and potassium (24.52 mg/100 g) were the most abundant minerals with appreciable amount of vitamin E and beta-carotene. Alkaloid (0.25%), saponin (10%), oxalate (0.015 mg/100 g) and phytate (0.487 mg/100 g) were found in the hull. It was therefore concluded that the findings of *M. oleifera* seed hull contain nutritive values that may necessitate its usage in alternative feed formulation instead of just remaining an agro waste.

Key words: Antinutrients, hull, minerals, *Moringa oleifera,* vitamins.

INTRODUCTION

Moringa oleifera is a widely cultivated medium sized tree species that originated from Northwest India. It is a fast growing aesthetically appealing tree characterised by long, drumstick shaped pods that contain about five to seven seeds within its first year of growth (Aja et al., 2013). Moringa tree grows in humid or hot dry lands and well adapted in less fertile soils and drought affected areas. In Nigeria, there is increasing awareness of the health and nutritional benefits of different parts of Moringa plant and government at various levels are now supporting the growth of this important tree (Bello et al., 2015).

The seed is composed of edible inner part covered with a round shaped semi permeable hull with whitish wings that run from top to bottom at an interval of 120°C. The seeds can be grounded into powder when dried and used as food or other purposes (Anjorin et al., 2010; Quattrochi, 1999).

M. oleifera seed hull does not seem to have any known uses and are generally peeled and dumped. Thus, this necessitates the study to provide relevant scientific data on the nutrient composition of *M. oleifera* seed hull.

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This work therefore aims at assessing the proximate composition, mineral and vitamin content and antinutrients of *M. oleifera* seed hull.

MATERIALS AND METHODS

Collection and sample preparation

Pods were collected from *M. oleifera* plantation in National Research Institute for Chemical Technology, Zaria. The hulls were removed from the seeds, dried at room temperature and then ground to a powder using mechanical grinder.

Proximate analysis

Crude protein was determined using Pearson (1976), while moisture content, crude fibre, lipids, and ash were determined using standard analytical procedures of AOAC (2000). Carbohydrate content was obtained by difference [100-% (protein+ash+lipid+moisture+fibre)].

Determination of minerals and vitamin contents

After digestion, sodium and potassium were determined using a GallenKamp Flame analyzer; Calcium, Magnesium, Iron, Zinc, and Copper were determined using Buch Model 205 Atomic Absorption Spectrophotometer (AAS); while the remaining mineral elements were determined by methods of Ruperez (2002). Vitamin C, vitamin E and beta-carotene contents were determined using UV-VIS spectrophotometer of Shimadzu.

Determination of antinutrients

Determination of oxalate

Titrimetric method of Munro and Bassir (1969) was used with slight modification. Exactly 75 cm^3 of 1.5 M H_2SO_4 was added to 1 g of the ground samples and the solution was carefully stirred intermittently with a magnetic stirrer for about 60 min and filtered. Then, 25 cm^3 of the filtrate was collected and titrated against hot (90°C) 0.1 M $KMnO_4$ solution until a faint pink colour that persisted for 30 s appeared. This was repeated two more times and the concentration of oxalate in each sample was obtained from the calculation: 1 cm^3 of 0.1 M $KMnO_4$ is equivalent to 0.006303 g Oxalate.

Saponin determination

Saponin was determined using the method reported by Ejikeme et al. (2014) and Obadoni and Ochuko (2002). Exactly 100 cm^3 of 20% C_2H_5OH(aq) was added to 5 g of each powder sample in a 250 cm^3 conical flask. The mixture was heated over a hot water-bath (55°C) for 4 h with continuous stirring. The residue of the mixture was re-extracted with another 100 cm^3 of 20% C_2H_5OH(aq) after filtration and heated at 55°C for 4 h stirring. The combined extract was evaporated to 40 cm^3 over water-bath at 90°C, where 20 cm^3 of diethyl ether was added to the concentrate in a 250 cm^3 separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice, as 60 cm^3 of n-butanol was added and extracted twice with 10 cm^3 of 5% NaCl. After discarding the NaCl layer, the remaining solution was heated in a water bath for 30 min, after which the solution was transferred into a crucible and was dried in an oven to a constant weight.

The saponin content was calculated as a percentage:

% Saponin = Weight of saponin / Weight of sample × 100

Phytic acid determination

Phytic acid was determined using the procedure described by Haug and Lantzsch (1983). A portion (2 g) of each sample was weighed into 250 cm^3 conical flask; 100 cm^3 of 2% concentrated HCl was used to soak each sample for 3 h. The mixture was then filtered and 50 cm^3 of each filtrate was placed in 250 cm^3 beaker, where 107 cm^3 of distilled water was added to each solution and titrated with standard $FeCl_2$ (aq) which contained 0.00195 iron per cm^3.

% Phytic acid = Y × 1.19 × 100

where Y = Titre value × 0.00195

Determination of alkaloid

The method reported by Ijarotimi et al. (2013) was adopted to determine the alkaloid content of the *M. oleifera* seed hull. Exactly 5 g of the sample was dispersed in acetic and ethanol (1:10) solution. The mixture was left for about 4 h at 28°C and filtered. The filtrate was evaporated and treated with concentrated $NH_4OH_{(aq)}$ drop wise to precipitate alkaloid. It was then washed in a pre-weighed filter paper and dried at 80°C in an oven and estimated.

Statistical analysis

All the analysis was carried out in triplicates and results expressed as mean ± standard error mean.

RESULTS

The proximate composition of *M. oleifera* seed hull is shown in Table 1. The hull contained crude protein 5.25±0.011%, fibre 19.5±0.038%, ash 5.0±0.026%, crude lipid 4.25±0.011%, carbohydrate 55.2±0.062 and moisture content of 10.8±0.033%.

The mineral composition of *M. oleifera* seed hull is shown in Table 2, where the most abundant minerals in the hull are sodium (45.49 mg/100 g) and potassium (24.52 mg/100 g).

The result of the vitamin content is shown in Table 3, with vitamin E (46.189 mg/100 g) having the highest value.

Alkaloid (0.25%), phytate (0.487mg/100 g), oxalate (0.015mg/100 g) and saponin (10%) were found in the hull of the seed (Table 4).

DISCUSSION

The crude fibre content of *M. oleifera* seed hull from this study is higher than 5.03±0.07% for *M. oleifera* seed reported by Ijarotimi et al. (2013), 11.40% and 15.34% in

Table 1. Proximate composition of *Moringa oleifera* seed hull.

Parameter	Proximate composition (%)
Moisture	10.8±0.033
Crude Fibre	19.5±0.038
Crude lipid	4.25±0.023
Crude protein	5.25±0.011
Ash	5.0±0.026
Carbohydrate	55.2±0.62

Expressed values were mean of three determinations ± their standard deviation

Table 2. Elemental analysis of *Moringa oleifera* seed hull.

Element	mg/100 g
Potassium	24.520
Sodium	45.490
Calcium	0.237
Iron	3.623
Magnesium	0.450
Copper	0.122
Cobalt	2.427
Lead	4.336
Cadmium	0.065
Manganese	0.210
Zinc	0.948
Nickel	0.836
Chromium	5.060

Table 3. Vitamin content of *Moringa oleifera* seed hull.

Parameter	Content (mg/100 g)
Beta-carotene	2.749
Vitamin C	0.698
Vitamin E	46.189

Table 4. Antinutrients content of *Moringa oleifera* seed hull.

Parameter (Unit)	Values
Alkaloid (%)	0.250
Saponin (%)	10.000
Phytate (mg/100 g)	0.487
Oxalate (mg/100 g)	0.015

Garcinia kola seeds and its hull, respectively (Eleyinmi et al., 2006) but lower than 83.22% in *Annona diversifolia* seed hull (Cuevas-Sanchez et al., 2011). The obtained value of crude fibre from this study is significant considering its possible health implication in the gastrointestinal tract of the animals (Bolanle et al., 2014). The carbohydrate and the crude fibre contents for instance may allow the hull to be used as alternative ingredient in feed formulation where necessary, except for the poor crude protein content.

The mineral composition findings agree with the report by Ijarotimi et al. (2013) who asserted that the most abundant mineral in raw *M. oleifera* seed was sodium (295.10±0.10 mg/100 g). Eleyinmi et al. (2006) also reported potassium as the most abundant mineral in *G. kola* seed hull. These two elements play a significant role in the nervous system and the regulation of pH and osmotic balance of fluids in the body. Iron (3.623 mg/100 g) was also detected in this hull, which is essential in the production of haemoglobin and as a critical cofactor in some metabolic reactions in animals (Ejidike and Ajileye, 2007).

The most abundant vitamin in *M. oleifera* seed hull was vitamin E (46 mg/100 g). Meanwhile, vitamin C (8.17 mg/100 g) was reported the most abundant in *Musa paradisiaca* Bract (Adeolu and Enesi, 2013). Vitamin E, a fat soluble antioxidant interferes with the propagation of reactive oxygen species (free radicals) that are implicated in disease conditions related to oxidative stress such as deficiencies in vitamin E which may result in nerve problems due to poor conduction of electrical impulses as well as weakness, weight loss and scurvy in vitamin C. *M. oleifera* seed hull has higher vitamins content when compared with cassava, *Magnifera indica* and *M. paradisiaca* (Adeolu and Enesi, 2013; Fowomola, 2010; Okigbo, 1980). Vitamins are needed in micro quantities but essential for healthy living. Also, vitamins have been shown to be effective in the damage of nucleic acid in pathogen that is found in plasma and platelets (Bruijn et al., 2010; Ruane et al., 2004).

The antinutrients result is similar to the findings from the work of Bolanle et al. (2014) who reported the presence of phytate in the seed of *M. oleifera* at a significantly low concentration. Presence of antinutrients in the *M. oleifera* seed hull is very minimal as compared to 2.0 to 2.4% phytate in *Cajanus cajan, Vigna ungiculata* and *Sphenostylis stenocarpa* (Onwuka, 2006). Phytate and oxalate react with divalent minerals such as calcium and magnesium to form complexes thereby hindering absorption, thus slowing down digestion.

Conclusion

There have been many researches on *M. oleifera* plants over the last few years, but its seed hull has been somehow neglected as an agro waste. This study has shown that *M. oleifera* seed hull may be a cheaply available alternative source of livestock feed formulation

to other costly feedstuff sources as a result of its satisfactory good nutritional qualities revealed from the data of this study. The satisfactory contents of crude fibre, carbohydrate, lipids, minerals and vitamins, as well as low antinutrients strengthen the implication that the seed hull may have positive health implication on animal if use for livestock feed formulation. Hence, further research is recommended on its nutritional evaluation to involve feeding tests and digestibility in animals for its inclusion in livestock feed formulation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 10(8), pp. 192-197, August 2019
DOI: 10.5897/IJLP2018.0532
Article Number: 5D47ED461619
ISSN 2141-2448

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Full Length Research Paper

Evaluation of varying levels of *Vernonia amygdalina* leaf meal on growth, hematological parameters and as anticoccidial

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Received 3 October, 2018; Accepted 26 June, 2019

Among the currently available poultry feed additives, natural herbs and plants have been widely advocated due to their reported widespread beneficial effects. *Vernonia amygdalina* is one of such potential feed supplements which have recently been reported as having a wide range of beneficial effects on production performance. This study was designed to evaluate the effect of varying levels of *V. amygdalina* leaf meal on growth performance, hematological parameters and as anticoccidial agent for broiler chicken. A total of one hundred and fifty day old marshal broiler chicks were randomly allotted to five dietary treatments with 30 birds per treatment, replicated thrice, in a completely randomized design. The treatments were: Treatment 1 (T_1) served as control (positive control) with inclusion of coccidiostat but no inclusion of *V. amygdalina*, T_2 served as negative control with no inclusion of either coccidiostat or *V. amygdalina,* T_3 (200 g of *V. amygdalina/* 150 kg of feed), T_4 (400 g of *V.amygdalinal* 150 kg of feed), and T_5 (600 g of *V. amygdalinal*150 kg of feed). Significant differences ($p < 0.05$) were observed in the growth performance characteristics. The result of hematological indices shows that there were no significant differences ($p > 0.05$) across the treatment except for hemoglobin in T_2 that has the lowest (8.04 g/dl) and T_4 that has the highest (12.03 g/dl). Eosinophil's in T_4 has the highest value (4.00%) and lowest in T_5 (2.00%) and T2 (2.00%). However, all other parameters were within the normal range. It can be concluded that *V. amygdalina* can be used as anticoccidial in broiler chickens due to high fed conversion ratio and Eosinophil's observed in T_3 (200 g) and T_4 (400 g), respectively.

Key words: Natural herbs, vernonia amygdalina, performance, haematological, coccidiostat.

INTRODUCTION

In order to improve poultry production and reduce losses due to outbreak and occurrence of disease, the use of antibiotics and anticoccidial has been on the increase with their residual effect. In an attempt to increase protein

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intake and reduce malnutrition, it is therefore suggested that expansion of this enterprise would help in feeding the expanding population with protein. Some growth promoters which are chemical and biological substances are added to poultry feed with the aim of improving the growth of chicken, improving the utilization of feed and in this way, realize better production (Dallouls et al., 2006). Their mechanism of action varies, but positive effect can be expressed through better appetite, improved feed conversion, stimulation of the immune system and increased vitality, regulation of the intestinal micro flora, etc. However, the continuous rise in cost of medicines (such as antibiotics) affects local broilers producers. The use of antibiotic growth promoters has been critized due to its possible role in the occurrence of antimicrobial resistance in humans. This new context caused an increase in the search for alternative growth promoters.

Vernonia amygdalina (VA) is a shrub or small tree that grows throughout tropical Africa. It is popularly called bitter leaf because of its abundant bitter taste (Ekpo et al*.,* 2007). The leaves contain a considerable amount of anti-nutritional factors like high level of tannic acid and saponin (Charles and Boulevard, 2012). The findings by Akwaowo et al*.* (2000) reported that the young leaves often preferred for human consumption contain high cyanide (60.1 mg 100^{-1}g DM) and tannin content (40. 6 mg 100^{-g} DM) than older ones. Furthermore, *V. amygdalina* has also been used as feed for broilers, where it was able to replace 300g kg^{-1}DM of maize based diet feed efficiency (Bonsi et al*.,* 1995). Research has shown that *V. amygdalina* have some beneficial effect in disease management of poultry (Dakpogan, 2006) such as anti-bacterial and anti-parasitic and anti-oxidant (Erasto et al*.,* 2009) and as growth promoter by enhancing the gastro intestinal enzymes thus increasing feed conversion efficiency (Huffinan et al., 1996; Olabatoke and Oloniruba, 2009).

The effects of any feed ingredient on the haematological factors of the chicken are of immense assistance in deciding whether or not such a feed ingredient will be used as poultry feedstuff (Mitruka and Rawsley, 1977). Certain heamatological factors such as packed cell volume, red blood cell, hemoglobin etc can be associated with certain production traits and serve as means of assessing clinical and nutritional health status of animals. For example it has been established that high percentage white blood cells especially lymphocytes are associated with the ability of the chicken to perform well under stressful conditions.

Coccidiosis is one of the most common diseases of poultry production systems in spite of advances in chemotherapy, management, nutrition and genetics (MC Dougald et al*.,* 1997). It remains a big concern to the commercial chicken production because of the high cost involves in the control of disease. Coccidiosis may strike any type of poultry in any type of facility (MC Dougald, 2003).The routinely use of these drugs on one hand has led to strains of parasites which are drug resistance (Long, 1986) and on the other hand, prejudicial to consumer health because of drug or antibiotic residue in poultry products (Youn and Noh, 2001). In recent years, interest has developed in many countries in the collection and extended use of medicinal plant extract for an alternative production purposes (Griggs and Jacob, 2005). The emergence of drug resistance strain of coccidial has made currently available anti coccidial less effective and this has threatened the economy of the country especially in developing countries where the problem has become a major concern to poultry farmers. However, this study has provided another alternative treatment of using *V. amygdalina* for coccidiosis which is targeted at solving farmers' problem. Therefore, the objective of the study is to evaluate the effects of varying levels of *V. amygdalina* leaf meal on growth, hematological parameters and as anticoccidial for broiler chickens.

MATERIALS AND METHODS

Experimental site and preparation of test ingredient

The experiment was carried out at the poultry experimental unit, Bora Farm of Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State, Nigeria. The *V. amygdalina* leaves were source around the school premises and air dried before milled into powdered form (0.84 mm).

Experimental birds, design and diets

One hundred and fifty day old marshal broiler chicks were purchased and allotted in five dietary treatments: T1, T_2, T_3, T_4 and T_5 with 30 birds per treatment and replicated thrice using completely Randomized design (CRD). Starter diet was served to the birds at the first to fourth week and finisher diet from fourth week to eight week` with inclusion of *V. amygdalina* (Bitter leaf) and coccidiostat as follows:

T_1- Positive control (they were naturally infected and treated under normal farm condition with the use of coccidiostat).
T_2- Negative control (they were naturally infected but neither treated with *V. amygdalina* or coccidiostat)
T_3- They were naturally infected and treated with the inclusion level of 200 g of *V. amygdalina*/150 kg of feed.
T_4- They were naturally infected and treated with the inclusion level of 400 g of *V. amygdalina*/150 kg of feed.
T_5- (they were naturally infected and treated with the inclusion level of 600 g of *V. amygdalina*/150 kg of feed)

Natural infection of experimental bird

Infected beddings of birds with coccidiosis were sourced from a farm that had an outbreak of coccidiosis. Thereafter, samples of the beddings were taken to the laboratory to confirm the presence of *Eimeria* which was confirmed positive. A week after brooding, the old litters of the experimental birds were packed and the infected litters were evenly spread across the treatment. At ten day of post infection, five gram of feacal samples were randomly selected across the treatment to confirm if the birds came down with the infection.

Table 1. Gross composition of experimental broilers starter diet.

Ingredient	Treatments				
	T_1	T_2	T_3 (200 g)	T_4 (400 g)	T_5 (600 g)
Maize	50.00	50.00	49.80	49.60	49.40
Vernonia	-	-	0.20	0.40	0.60
Soya bean meal	30.00	30.00	30.00	30.00	30.00
Wheat offal	11.00	11.00	11.00	11.00	11.00
Fish meal	4.00	4.00	4.00	4.00	4.00
Bone meal	4.50	4.50	4.50	4.50	4.50
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0,10	0,10	0,10	0,10	0,10
Salt	0.30	0.30	0.30	0.30	0.30
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Crude protein (%)	22.46	22.46	22.46	22.46	22.46
Crude fiber (%)	4.00	4.00	4.00	4.00	4.00
Ether extract (%)	4.00	4.00	4.00	4.00	4.00
M. E (kcal/kg)	2847.10	2847.10	2847.10	2847.10	2847.10

Determination of coccidial infection

At ten day of post infection, 5 g of feacal sample was randomly selected from each treatment and taken to the laboratory for feacal parasite screening using floatation method.

Management of experimental birds

The cages were washed and disinfected using Morigad disinfectant and left to rest for two weeks prior to the arrival of the chicks. Before the arrival of the birds, the brooding cages were ready and water was waiting for them upon arrival. The birds were raised on a normal starter diet for 4 weeks and finishers' diet for four weeks. Vaccination was carried out as at when due, while routine medication was done to keep the birds healthy except giving them Coccidiostat. Water and feed were served at *ad-libitum* before the birds were separated into different treatments with their feeds weighed out on daily basis. Leftovers were weighed and recorded and subtracted from the feed giving to determine the feed intake.

Blood collection and haematology analysis

At the end of sixth week of feeding trials, two birds from each dietary replicate were randomly sampled to determine haematological responses. 5 ml of blood was taken from the jugular vein of randomly selected birds per replicate. 2.5 ml of sampled blood was put into labelled blood sample bottles containing anti-coagulant (Ethyl Diamine-Tetra-Acetate powder (EDTA)) to determine haematological parameters. Parameters analysed include; Packed Cell Volume (PCV)(%), Haemoglobin (g/l), Red Blood Cells (RBC)(10^6ul), White Blood Cell (WBC)(10^3ul), Lymphocytes (%), Neutrophils (%), Monocytes (%), Eosinophil (%) and Basophils (%) according to the procedure of Howlett and Jamie (2008).

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) according to the procedure of SAS (2002). Significant differences between the treatments means were separated using Duncan multiple range test (Duncan, 1955).

Data collection

Initial weight of birds was taken before they were distributed into various treatments. Experimental diet containing *V. amygdalina* was served over a period of 7 weeks. Thereafter, final weights were determined using the kitchen weighing scale. The total feed intake was calculated over a period of 7 weeks. Feed intake (g) is the daily feed consumed which was obtained by deducting the weight of remnant feed from the feed offered the previous day. Body weight gain (g) was done on weekly basis. It was obtained by deducting the previous week's body weight from the subsequent week's body weight.

RESULTS AND DISCUSSION

Tables 1 and 2 show the proximate analysis of the experimental broiler starter and finisher diets. Both starter and finisher are isocaloric and isonitrogenous in nature. Table 3 shows the phytochemical constituents of the test ingredients. The levels of tannin, phenol, steroid, Phytate and oxalate levels are moderately positive while that of alkaloid, saponin, flavonoids and cyanide are strongly positive. Table 4 shows the performance characteristics of broiler fed diet with varying levels of *V. amygdalina*. The highest final weight was recorded in T2 (1591.17 g) and lowest in T5 (1196.83 g). The highest final weight gain was observed in T2 (1466.40 g) and the lowest in T5 (1062.40 g). For average daily weight gains, highest value was recorded in T2 (29.92) while the least value was obtained in T5 (21.68). The result for feed intake ranges from highest T1 (3362.00 g) to lowest T5

Table 2. Gross composition of experimental broilers finisher diet.

Ingredient	Treatments				
	T_1	T_2	T_3 (200 g)	T_4(400 g)	T_5 (600 g)
Maize	55.88	55.88	55.68	55.48	55.28
Vernonia	_	_	0.20	0.40	0.60
Soya bean meal	24.42	24.42	24.42	24.42	24.42
Wheat offal	13.00	13.00	13.00	13.00	13.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Crude protein (%)	20.00	20.00	20.00	20.00	20.00
Crude fiber (%)	4.00	4.00	4.00	4.00	4.00
Ether extract (%)	4.00	4.00	4.00	4.00	4.00
M. E (kcal/kg)	2700.00	2700.00	2700.00	2700.00	2700.00

Table 3. Phytochemical constituent of *V. amygdalina (*Bitter leaf).

Parameter determined	Qualitative	Quantitative
Tannin (mg/100 g)	++	0.422
Phenol (mg/100 g)	++	0.316
Alkaloids (mg/100 g)	+++	0.425
Saponin (mg/100 g)	+++	0.573
Flavonoids (mg/100 g)	+++	0.483
Terpenes (mg/100 g)	+	0.212
Steroid (mg/100 g)	++	0.174
Phytate (mg/100 g)	++	0.366
Oxalate (mg/100 g)	++	0.244
Cyanide (mg/100 g)	+++	0.134

+ = Positive; ++ = Moderately positive; +++ = Strongly positive.

(2876.34 g). For feed conversion ratio, lowest value was recorded in T2 (1.99) to the highest of (2.40) T5. The mortality percentage reveals that T2 had the highest percentage of 20% while T5 has 6% mortality while other treatment had no mortality.

The result of the hematological parameters of broiler chicken fed varying levels of *V. amygdalina* based diet is as shown in Table 5. The result of hematological indices shows that there were no significant differences across the treatment except for hemoglobin in T2 that had the lowest value (8.04 × g/dl) while T4 had the highest (12.03 × g/dL). Eosinophil in T4 has the highest value (4.00%) and lowest in T5 (2.00%) and T2 (2.00 %). Packed cell volume in T4 has the highest value (38.00 %) and lowest in T2 (25.00%). Red blood cell was higher in T3 (3.57×10^{12}/L) and lower in T2 (2.43×10^{12}/L). However, all other parameters were within the normal range recommended by Merck (2011).

DISCUSSION

The result shows the performance characteristics of finisher broiler diet containing varying levels of *V. amygdalina* based diet. From this result it was observed that birds in T_2 (negative control) and T_1 (positive control) had the best result in terms of feed intake, final weight, weight gain, average daily weight gain and the best feed conversion ratio. This could be as a result of non - inclusion of *V. amygdalina* leaf meal in the diet of these

Table 4. Performance of broiler chicken fed *V. amygdalina* based diet.

Parameter	Treatments					
	T_1	T_2	T_3 (200 g)	T_4 (400 g)	T_5 (600 g)	Sem
Initial weight(g)	125.77	125.07	122.80	133.53	134.43	2.00
Final weight(g)	1498.67^{ab}	1591.17^{a}	1493.00^{b}	134383^{bc}	1196.83^{c}	43.94
Weight gain(g)	1372.90^{ab}	1466.10^{a}	1370.20^{b}	1210.30^{bc}	1062.40^{c}	45.34
Average daily weight gain(g)	28.02^{ab}	29.92^{a}	27.96^{ab}	24.70^{b}	21.68^{c}	0.93
Feed intake(g)	3362.00^{a}	3169.96^{b}	3215.04^{ab}	3144.24^{b}	2876.34^{bc}	41.09
Feed conversion ratio (%)	2.24^{bc}	1.99^{c}	2.15^{bc}	2.33^{b}	2.40^{c}	0.08
Mortality (%)	0.00	20.00	0.00	0.00	6.00	

$^{a.b.c}$ (superscript) means of different superscription along the same row are significantly different ($p<0.05$).

Table 5. Hematological parameters of broiler chicken fed varying level of *V. amygdalina* based diet.

Parameter	Treatments					*Range
	T1	T2	T3	T4	T5	
Packed cell volume (%)	35.00	25.00	37.00	38.00	33.00	24.9-45.20
Hemoglobin (g/dl)	11.06	8.04	12.03	12.03	11.00	7.40-13.10
Red blood cell (10^{12}/L)	3.54	2.43	3.57	3.51	3.46	1.58-4.10
White blood cell (10^{12}/L)	13.65	15.00	12.25	15.60	11.40	9.20-31.00
Platelets (%)	20.80	22.00	21.10	29.20	22.00	15.6-60.40
Lymphocyte (%)	72.00	68.00	70.00	66.00	64.00	43.90-67.70
Heterophils (%)	21.00	24.00	24.00	25.00	31.00	25.2-35.10
Monocyte (%)	4.00	5.00	3.00	2.00	3.00	0.06-9.10
Eosinophil's (%)	3.00	2.00	3.00	4.00	2.00	6.25-9.66
Basophils (%)	0.20	1-00	0-15	1.00	0.16	2.50-5.36

*Mitruka and Rawsley (1977).

treatments and this might have resulted in better performance observed. Highest mortality ratio was recorded in T_2, which must have be as a result of non - inclusion of *V. amygdalina* or the use of anticoccidial drugs which must have led to outbreak of coccidiosis. Comparing the varying levels of inclusion, in terms of final weight, weight gain, average daily weight gain and feed intake, T_3 (200g) has the best result. T_3 (200g) also has the lowest feed conversion ratio value among the three inclusions levels and hence the treatment with best feed conversion. This could be as the result of the low concentration of the leaf meal which might have improved the performance. Lowest feed intake, average daily weight gain, weight gain and final weight were recorded in T_5 (600g). This might be as a result of higher concentration of anti-nutritional factors such as alkaloid, saponin, tannin and glycoside in *V. amygdalina* as reported by Arhoghro et al., (2009). No mortality was observed from the birds on T1, T3 and T4 while 6% mortality rate was recorded in T_5 (600g). This indicates that as the level of inclusion of bitter leaf increased the mortality rate increased. This could be as a result of the anti - nutritional factors in the test ingredient (Saponin, Alkaloids and Tannin).

The result of hematological parameters of broilers birds fed diet with varying inclusion levels of *V.amygdalina* shows that bird fed with inclusion level of 400g (T_4) has highest packed cell volume. For hemoglobin, T_3 and T_4 with inclusion level of 200 and 400 had the highest value also. For red blood cell, white blood cell T_3 with inclusion level of 200 g has higher value. Owen and Amakiri (2011) made similar observation, with the exception of White blood cell. All other hematological indices measured were influenced by increasing levels of *V. amygdalina*. Osho et al. (2014) demonstrated in his own experiment that oral adminstation of bitter leaf extract on broiler chickens did not have a significant effect on the Hb and RBC in treated birds. Increase in White blood cell in T_4 could be attributed to the presence of anti-nutritional compounds of *V. amygdalina* and presence of infection. This is in line with Aregheore et al. (1998) who suggested that the presence of some phytochemicals in bitter leaf extract allows the animal to respond to infection. According to Isaac et al. (2013), packed cell volume is involved in the transport of oxygen and absorbed nutrients. Increased packed cell volume shows a better transportation and thus prevents anaemia (Coles, 1986). The result of packed cell volume and haemoglobin which

increases as the inclusion level of bitter leaf increased in the diets is in accordance with Adejumo (2004) who reported that packed cell volume and haemoglobin were positively correlated with the nutritional status of the animal. This observation however implies that the diets supported haemopoictic tissue with production of adequate white blood cells. Thus result indicated that the immune system of the birds was not compromised because the White Blood Cells function primarily as defence system in the body (Eroschenko, 2000). Values obtained for Neutrophils, Monocytes Eosinophils and basophiles are within the normal range for healthy birds (Mitruka and Rausley, 1977; Archetti et al*.,* 2008).

It was reported by Frandson (1986) that the number of neutrophils in the blood increases rapidly when acute infection is present; hence a blood count showing this increase is useful in diagnosis of infections, which is contrary to this result. The low values of monocytes and basophils agreed with the statement that basophils and monocytes are normally present in small to moderate number in the blood system. For Eosinophil, T_4 has the highest value, and eosinophil is known to protect animal against infection. This shows birds on this treatment were able to fight against the infection. Other treatments were also high but not as high as T_4.

Conclusion

Based on the study, it can be concluded that inclusion of *V. amygdalina* at the rate of 200 g - 400 g/ 150 kg feed had no adverse effect on the growth and hematological parameters measured; it also prevented the occurrence of coccidiosis in broiler chicken. Hence, the addition of this natural herb at this level, 200 g - 400 g/150 kg of feed can be used as anticoccidial in broiler chickens' diet without any delecterious effect on the health status of the birds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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