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ORAL ADMINISTRATION OF *Vernonia amygdalina* LEAF EXTRACT: IMPLICATIONS ON PERFORMANCE OF BROILER FINISHERS RAISED IN DERIVED SAVANNAH

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ABSTRACT

A four-week study was conducted to investigate the effect of Vernonia amygdalina leaf extract (VALE) on growth, haematology and biochemical indices of broiler finisher birds. A total of 180 four-week old Arbor acre strain commercial broilers were randomly selected and assigned to four experimental treatments namely; T1-0 ml VALE (control), T2-20 ml VALE per liter of water, T3-40 ml VALE per liter of water, T4-60 ml VALE per liter of water. Daily feed and water intake were recorded. Weekly body weight was taken and used to calculate feed: gain ratio. At the end of the experiment, blood samples were collected from twelve broilers per treatment for haematological and serum biochemical studies. Results showed that broilers on T3 (40 ml VALE) and T4 (60 ml VALE) had significantly (P < 0.05) reduced daily feed and water intake but had a better average daily weight gain and feed: gain ratio (P < 0.05). The haematological indices of the birds showed significant (P < 0.05) differences in haemoglobin, pack cell volume, and white blood cell counts in favour of birds in T3 and T4. The biochemical indices showed no differences (P > 0.05) in aspartate transaminase, alanine transaminase, alkaline phosphatase, total protein and globulin levels. Moreover, cholesterol, triglycerides and low-density lipoprotein levels of birds on T3 and T4 were significantly (P < 0.05) reduced compared to those on T1 (0 ml VALE) and T2 (20 ml VALE), while albumin and high-density lipoprotein levels were significantly (P < 0.05) increased. The study showed that oral administration of 40 and 60ml VALE improved performance of birds and had no deleterious effects on haematological and serum biochemical indices of birds. Therefore, it can be used as nutrient supplement in poultry production.

Key words: broiler finishers, *Vernonia amygdalina,* growth, haematology, biochemical indices

INTRODUCTION

The public and scientific alarm about the prevalent application of antibiotics and the possible development of both drug resistance, cross resistance and multiple resistance to human pathogenic bacteria necessitated the ban for non-therapeutic purposes (Osita *et al.,* 2019). Hence, the increased effort by researchers to find safe alternatives to the use of antibiotics in livestock production (Machebe *et al.,* 2011; Osho *et al.*, 2014; Oleforuh-Okoleh *et al*., 2015; Chiemela *et al*., 2016; Ndelekwute *et al*., 2017; Okunlolo *et al.*, 2018). *Vernonia amygdalina,* peculiarly called bitter leaf (English) is a medicinal plant with antimicrobial properties that is widespread in East and West Africa (Ndelekwute *et al*., 2017). The leaves have been identified to be used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort, among others. (Usunobun and Okolie, 2016). Huffman *et al.* (1996) suggested that the bitterness of *Vernonia amygdalina* might enhance the gastrointestinal enzymes especially chymotrypsin production which in turn might enhance the digestion of sporozoites. Research has observed that *Vernonia amygdalina* has some potential in disease management of poultry including serving as anti-coccidiosis, anti-bacterial, and anti-parasitic (Dakpogan, 2006; Olobatoke and Oloniruha, 2009); as an anti-oxidant (Erasto *et al*., 2007); and as a growth promoter by enhancing the gastrointestinal enzymes thus improving feed conversion efficiency (Oleforuh-Okoleh *et al*., 2015).

Against the above backdrops, the present study was designed to evaluate the efficacy of oral administration of *Vernonia amygdalina* leaf extract (VALE) on growth performance, haematological and biochemical indices of broiler finisher birds reared in derived savannah zone.

MATERIALS AND METHODS

The study was carried out at the Poultry Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka, Enugu State. Nsukka lies in the derived savannah region at between longitude 6°51'24'N and latitude 7°23'45'E (http://thegpscoordinates.net/nigeria/nsukka). The average rainfall, temperature and relative humidity in Nsukka were 1579 mm, 24.9°C, and 75% respectively (climate-data.org, 2016). The study lasted for four weeks. The experimental procedures complied with the provisions of the University of Nigeria, Nsukka Ethical Committee on the Use of Animals for Biometric Research.

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Collection and Preparation of *Vernonia amygdalina* Leaf Extract (VALE)

Fresh leaves of *Vernonia amygdalina* were bought from the local market in Nsukka, Enugu State, Nigeria. The leaves were washed thoroughly without squeezing with clean tap water to remove dirt and sand. They were later air-dried under room temperature for 10 days. The dried leaves were later hammer milled into tiny particles and subsequently analysed for proximate composition (AOAC, 2000) and some phytochemical compositions using methods reported by Okunlolo *et al.* (2018). A total of 100 g of the milled *Vernonia amygdalina* leaves was infused into 1 litre of hot boiled water for 12 h (overnight). The soaked leaves were then sieved and the supernatant collected were stored in a refrigerator maintained at 5^{o}C and later used daily as *Vernonia amygdalina* leaf extract (VALE). This protocol was repeated every three days in order to ensure the availability of fresh samples of the extract.

Experimental Procedure

A total of 200-day old Arbor Acres commercial broiler chicks were brooded for four weeks after which 180 birds were randomly selected and assigned to four experimental treatments in a completely randomized design (CRD). Each experimental treatment contained 45 birds made up of three replicates of 15 birds each. The treatments were T1 (water only), T2 (20 ml per liter of water), T3 (40 ml per liter of water), and T4 (60 ml per liter of water). Birds in T1 served as the control. Feed and water were supplied *ad-libitum*, while other standard broiler management procedures were meticulously followed throughout the duration of the study. The birds were weighed at the beginning of the experiment and weekly thereafter. The compositions of the experimental diet are shown in Table 1.

Table 1: Percentage and proximate composition of experimental diet

Ingredient	Composition (%)
Maize	30.00
Wheat offal	8.10
Cassava root meal	16.50
Groundnut cake	24.30
Palm kernel cake	12.10
Fishmeal	4.00
Bone meal	4.00
Methionine	0.25
Lysine	0.25
Salt	0.25
Vitamin/mineral premix	0.25
Total	100
Proximate	Dry matter (%)
Crude protein	22.00
Crude fibre	5.80
Ether extract	9.90
Ash	6.80
Metabolisable Energy	3001.00 ME kCal/kg

Data on growth performance were obtained by records from the daily water intake (i.e., volume of water given minus volume of water leftover), feed intake (i.e., quantity of feed given the birds minus quantity of feed leftover), and weekly weight. At the end of the experiment, these were used to calculate the weight gain and feed conversion ratio (feed consumed divided by weight gain).

Blood Collection for Analysis

At the age of eight (8) weeks, a total of 48 birds (four birds per replicate per treatment) were randomly selected and bled by severing the jugular vein. Procedures for slaughtering and blood collection were in line with approved guideline for humane treatment of animals (FASS, 1999). Blood samples were collected by allowing the blood to flow into labelled bottles containing EDTA as anticoagulant. These were used to determine the haemoglobin, packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), and the white blood cell count differentials according to Jain (1986). Another blood was collected from the birds without anticoagulant into a vacutainer to determine the following serum biochemical indices; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein (TP), albumin, globulin, cholesterol, triglycerides, high density lipoproteins (HDL), and low-density lipoproteins (LDL) level in the blood.

Statistical Analysis

Data were subjected to one-way analysis of variance using SPSS 16.0 computer statistical package in accordance with a completely randomized design (CRD). Significant differences among the treatment means were separated using the Duncan multiple range test (Duncan, 1955) at 5% level of probability.

RESULTS

Some determined chemical constituents of the air-dried *Vernonia amygdalina* leaves used are shown in Table 2. The proximate composition showed relatively high level of crude protein, carbohydrates, while crude fat/oil were of relatively low levels. The level of alkaloids, phenol, tannin at 7.4%, 2.5 mg/100g, 2.00% respectively were relatively substantial, while cynogenic glycosides were present at a low level.

Table 2: Proximate and phytochemical composition of air-dried *Vernonia amygdalina* leaves

Ingredient	Composition (%)
Crude protein	32.60
Crude fiber	9.55
Crude fat/oil	4.80
Ash	11.00
Carbohydrate	32.98
Phytochemicals	
Alkaloids (%DM)	7.40
Phenol (mg/100g)	2.50
Tannin (%DM)	2.00
Cynogenic glycoside (mg/100g)	0.20

Growth Performance Traits

The growth performance of broiler finisher birds administered oral VALE is presented in Table 3. The results depict that there were significant ($P < 0.05$) differences observed in final body weight, weight gain, feed intake, water intake and feed conversion ratios among the treatment groups. Broiler finisher birds on the control group had similar ($P > 0.05$) final body weights and daily weight gains with those administered 20 ml VALE per liter of water. However, they were significantly ($P < 0.05$) lower than those of the birds administered 40 and 60 ml per liter of water. The daily feed intake of the control finisher birds was not significantly ($P > 0.05$) different from those on 20 and 40 ml VALE per liter of water but were significantly ($P < 0.05$) higher than those of birds administered 60ml VALE per liter of water. On the other hand, birds administered VALE (20, 40, and 60 ml per litre of water) had similar ($P > 0.05$) daily water intake but significantly ($P < 0.05$) lower than the control. Feed conversion ratio was calculated as daily feed consumed per unit daily gain. The feed conversion ratio of broiler finishers given water only (the control) and those administered 20 ml VALE per liter of water were similar ($P > 0.05$) but significantly ($P < 0.05$) different from those orally given 40 and 6 0ml VALE per liter of water (Table 3).

Haematological Indices

The haematological indices of broiler finisher birds administered oral VALE are shown in Table 4. The results of the present study showed that there were no significant ($P > 0.05$) differences among the treatment groups for RBC values of the birds. Consequently, it was found that oral administration of VALE influenced significantly ($P < 0.05$) the haemoglobin, Pack cell volume, white blood cell count as well as the differential white blood cell counts. The haemoglobin and packed cell volume (PCV) of the finisher birds fed 40 and 60 ml VALE per liter of water were similar ($P > 0.05$) but significantly ($P < 0.05$) higher than those of the birds fed water only (control) and 20 ml VALE. The mean WBC content of the blood of finisher birds on water only (control) were significantly ($P > 0.05$) lower than those administered 20, 40 and 60 ml oral VALE which were similar ($P > 0.05$). The white blood cell differentials showed that finisher birds given water only and 20 ml VALE per liter of water were not statistically different ($P > 0.05$). However, their values differed significantly ($P < 0.01$) from those on 40 and 60 ml VALE.

Biochemical Indices

Table 5 shows the biochemical indices of finisher broiler birds administered oral VALE indicating that AST, ALT, ALP, TP and globulin levels in the blood were not significantly ($P > 0.05$) affected by the oral administration of VALE. Moreover, plasma albumin content of finisher broiler birds administered 40 and 60 ml VALE per liter of water were significantly ($P < 0.05$) higher than those of the control birds and T2 (20 ml of VALE). The results of the lipid profile of the birds showed that cholesterol, triglycerides, HDL and LDL levels in the blood were significantly ($P < 0.05$) influenced by VALE. The lipid profile (cholesterol, triglycerides, and LDL) of finisher broiler birds on 40 and 60 ml VALE per litre of water were significantly reduced compared to those of the birds fed 20 ml VALE and water only. Furthermore, the HDL levels in the blood were increased ($P < 0.05$) among finisher broiler birds on 40 and 60 ml VALE per litre of water (Table 5).

Table 3: Growth performance of broiler finisher birds administered oral VALE

Parameter	T1 (Control)	T2 (20 ml VALE)	T3 (40 ml VALE)	T4 (60 ml VALE)	SEM	*P* value
IBW (g)	927.29	926.81	921.91	925.71	18.73	0.76^{NS}
FBW (g)	2339.21^{b}	2414.29^{b}	2547.61^{a}	2577.78^{a}	29.34	0.03*
TWG (g)	1411.92^{b}	1487.48^{b}	1625.70^{b}	1652.07^{a}	32.28	0.02*
DWG (g)	50.43^{b}	53.12^{b}	58.06^{a}	59.00^{a}	1.15	0.02*
DFI (g)	180.80^{a}	179.70^{a}	170.04^{ab}	160.24^{b}	2.77	0.00**
DWI (ml)	549.41^{a}	493.34^{b}	478.70^{b}	475.30^{b}	8.61	0.00**
FCR	3.58^{a}	3.38^{a}	2.93^{b}	2.75^{b}	0.08	0.00**

IBW; Initial body weight, FBW; Final body weight, TWG; Total weight gain, DWG; daily weight gain, DFI; daily feed intake, DWI; daily water intake, SEM; Standard error of the mean, NS; not significant

^{ab}: Means on the same row with different superscripts are significantly different ($P < 0.05$ or $P < 0.01$)

Table 4: Haematological indices of Broiler Finisher Birds administered oral VALE

Parameter	T1 (Control)	T2 (20 ml VALE)	T3 (40 ml VALE)	T4 (60 ml VALE)	SEM	*P* value
Haemoglobin (g/dl)	8.09^{b}	8.26^{b}	9.87^{a}	9.64^{a}	0.11	0.00**
Pack cell volume (%)	25.50^{b}	26.00^{b}	30.50^{a}	29.50^{a}	0.40	0.00**
RBC($x10^{6}/mm^{3}$)	4.67	4.60	4.45	4.41	0.05	0.11^{NS}
WBC ($x10^{3}/mm^{3}$)	12.25^{b}	15.88^{a}	17.25^{a}	17.70^{a}	0.47	0.00**
Neutrophils (%)	52.50^{b}	52.00^{b}	60.50^{a}	59.50^{a}	4.22	0.00**
Lymphocytes (%)	34.50^{b}	35.50^{b}	37.00^{a}	37.50^{a}	4.67	0.00**
Monocytes (%)	8.50^{a}	8.50^{a}	1.50^{b}	2.00^{b}	0.28	0.00**
Eosinophils (%)	4.00^{a}	3.50^{a}	1.00^{b}	1.00^{b}	0.31	0.00**
Basophils (%)	0.67^{a}	0.67^{a}	0.00^{b}	0.00^{b}	0.09	0.00**

RBC: red blood cell count; WBC: white blood cell count, NS; not significant

^{ab}: Means on the same row with different superscripts are significantly different ($P < 0.05$ or $P < 0.01$)

Table 5: Biochemical indices of finisher broiler birds administered oral VALE

Parameter	T1 (Control)	T2 (20 ml VALE)	T3 (40 ml VALE)	T4 (60 ml VALE)	SEM	*P* value
AST (iu/l)	56.00	55.67	55.00	55.33	5.32	0.73 ^{NS}
ALT (iu/l)	64.00	63.00	63.00	62.67	4.94	0.61 ^{NS}
ALP (mg/dl)	34.50	34.55	35.00	35.00	3.18	0.82 ^{NS}
TP (g/l)	40.00	41.33	42.06	43.86	4.31	0.23 ^{NS}
Albumin (g/l)	20.12^{b}	21.69^{b}	22.76^{a}	23.12^{a}	2.76	0.04*
Globulin (g/l)	19.88	19.44	19.27	20.74	3.14	0.12 ^{NS}
Cholesterol (mmol/l)	4.60^{a}	4.38^{a}	3.90^{b}	3.60^{b}	0.30	0.02*
Triglycerides (mmol/l)	1.55^{a}	1.35^{a}	0.95^{b}	0.80^{b}	0.20	0.01**
HDL (mmol/l)	1.02^{b}	1.10^{b}	1.35^{a}	1.38^{a}	0.05	0.04*
LDL (mmol/l)	3.80^{a}	3.75^{a}	3.35^{b}	3.30^{b}	0.25	0.04*

ALT; alanine transaminase, AST; aspartate transaminase, ALP; alkaline phosphatase, TP; total protein, HDL; high-density lipoproteins, LDL; low-density lipoproteins, NS; not significant

^{ab}: Means on the same row with different superscripts are significantly different ($P < 0.05$ or $P < 0.01$)

DISCUSSION

The air dried *Vernonia amygdalina* leaves used for this study had crude protein value of 32.60%, which was higher than the values (22.10%) reported by Usunobun and Okolie (2016) but was in agreement with the values (17 to 33%) reported by Yeap *et al*. (2010). Proteins are considered building blocks and are important components of antibodies, hormones, digestive enzymes, as well as involved in structural support (Usunobun and Okolie, 2016). The crude fibre value of 9.55% (Table 2) obtained in this study was in agreement with the report of Yeap *et al.* (2010) but was higher than 3.83% by Okunlolo *et al.* (2018). The differences may be attributed to possible differences in varieties or time of harvest. The crude fat and ash values of 4.80% and 11.0% respectively were in agreement with the values of Yeap *et al.* (2010). Ash content is a reflection of the mineral contents preserved in the plant leaves and therefore suggests a considerably high deposit of mineral elements in the air-dried *Vernonia amygdalina* leaves (Lockett *et al.,* 2005). The carbohydrate content value (32.98%) of the air dried *Vernonia amygdalina* leaves used for this study was comparable to the values of Usunobun and Okolie (2016) and Okunlolo *et al*. (2018) who reported 38.03% and 40.47%, respectively. Carbohydrates produced by plants are one of the three main energy sources in food, along with protein and fat. Thus the values obtained shows a significant contribution to the energy value of *Vernonia amygdalina* leaves.

Phytochemicals are natural occurring substances found in plants and they are beneficial for health, because they protect against free radicals and possess antiviral and antibacterial properties (Manach *et al.,* 2004; Khan *et al*., 2016). Alkaloids have a strong bitter taste and confers *Vernonia amygdalina* leaves with its bitter taste property while polyphenols have been reported to have high antioxidant activities (Okunlolo *et al*., 2018). Antimicrobial, as well as many physiological activities such as stimulation of phagocytic cells, host-mediated tumor activity and a wide range of anti-infective actions have been attributed to tannins (Haslam, 1996). The presence of tannins and alkaloids as shown in our results (Table 2) are similar to the findings of Usunobun and Okolie (2016) who applied the standard phytochemical screening methods to identify the presence or absence of tannins and alkaloids in *Vernonia amygdalina*.

Machebe *et al.* (2011) opined that growth is an indication of the quality and utilization of available nutrient. Thus, it may be inferred that the addition of VALE increased the ability of broiler finishers to utilize available nutrients in the feed. The high protein content level of 32.60% obtained in the dried *Vernonia amygdalina* leaves could also contribute to the improved quality of feed utilization by the birds fed 40 and 60 ml VALE per litre of water. The results of this study are comparable with those of Osho *et al.* (2014) and Chiemela *et al.* (2016) who reported improved growth performance of birds fed *Vernonia amygdalina* meal. The reduced feed and water intake with increasing VALE inclusion could be as a result of the bitter taste of the VALE which made birds to consume less feed and water. The air-dried *Vernonia amygdalina* contained significant amounts of alkaloids which were responsible for the bitterness, thereby reducing appetite to ingest feed. Olobatoke and Oloniruha (2009) reported that the bitter taste of *Vernonia amygdalina* and the presence of anti-nutritional factors lowers intake of feeds in which it is incorporated. However, the feed conversion ratio of birds improved with the administration of 40 and 60 ml VALE per litre of water. This may be because *Vernonia amygdalina* has been reported to enhance the production of gastrointestinal enzymes which in turn may improve the utilization of feed (Huffman *et al.,* 1996). Mohammed and Zakariya'u (2012) also reported improved feed conversion ratio of broilers fed 900 g supplemental *Vernonia amygdalina.*

Haematological indices are often used for the diagnosis and treatment of animal diseases. The values (Table 4) obtained in this study were within normal ranges for broilers signifying that there were no symptoms of physiological anemia in the experimental birds (Al-Nadewi, 2018). The haemoglobin and PCV values were higher ($P < 0.05$) in broilers administered 40 and 60 ml VALE per

litre of water. Haemolysis results from acute inflammation from most pathogenic microorganisms which is manifested in lower haemoglobin level and PCV (Osho *et al.,* 2014). Thus, the improved values in finisher birds fed with 40 and 60 ml VALE per litre of water could be due to the inability of these treatments to cause haemolysis, resulting from the anti-inflammatory potentials inherent in *Vernonia amygdalina.* Moreso, the results of this study suggest that the VALE has the potential to act as an immuno-stimulant through the alteration of the occurrences and distribution of the white blood cell counts and the differentials. The improved neutrophils and lymphocytes percentage could be an indication of improved immune response (Stewart and Weir, 1997; Osho *et al.,* 2014). Other reports by Olobatoke and Oloniruha (2009) and Machebe *et al*. (2011) show that antioxidant phytochemicals play a protective role on the lymphocytes and reduce their destruction in the blood.

The aminotransferases, AST and ALT are typically inside the liver cells and are released into the blood when liver cells are damaged; increased levels are therefore indicative of liver damage (Weignand *et al.,* 1996). The findings of this study showed that oral administration of VALE did not enact liver damages as shown in statistical similarity ($P > 0.05$) when compared to the control group (Table 5). Total protein is a composite of albumin and globulin contents in the blood and are often used in diagnosing diseases and monitoring changes in the health status of farm animals (Oleforuh-Okoleh *et al*., 2015). Low levels of albumin could be associated with presence of infection. The total protein of finisher birds in this study were not influenced by VALE. Moreover, albumin levels were improved among birds given 40 and 60 ml VALE per litre of water. Similar results were reported in the findings of Oleforuh-Okoleh *et al*. (2015). Levels of cholesterol, triglycerides and LDL were reduced ($P < 0.05$) among birds given 40 and 60 ml VALE per litre of water whereas they experienced increased HDL levels (Table 5). This could be as a result of the reduced triglyceride biosynthesis and the favored redistribution of cholesterol among the lipoprotein molecules. Also, one of the key elements of danger for cardiovascular disease is a low HDL level (Assman and Gotto, 2004). The elevated levels of HDL in the 40 and 60 ml VALE birds is an indication of the protective role against cardiovascular diseases (Nofer *et al.*, 2002). Studies have shown that HDL promotes the reverse cholesterol pathway by inducing an efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL (Yokozawa *et al.,* 2006). Evidence from our study is similar to those of Adaramoye *et al*. (2008) and Owen *et al*. (2011) who reported lipid lowering effects of bitter leaf in rats and broilers, respectively.

CONCLUSION

This study has brought to the fore the potential of oral administration of *Vernonia amygdalina* leaf extract since the review of literature produced majorly reports of inclusions in feeds of poultry. The study revealed that *Vernonia amygdalina* leaf extract at 40 and 60ml per liter of water improved daily weight gain and feed conversion ratio. The study also showed that *Vernonia amygdalina* leaf extract had no toxic effect on the haematological and serum biochemical indices of broiler finisher birds. This is mostly significant today when emphasis is on increasing indigenous contents in production.

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