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Growth performance, haematological and biochemical parameters in broilers fed diets with varying levels of *Vernonia amygdalina* leaf meal

Einfluss unterschiedlicher Konzentrationen von *Vernonia amygdalina*-Blattmehl im Futter auf Wachstumsleistung, hämatologische und biochemische Parameter bei Masthühnern

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Abstract

Growing concern about the use of antibiotics as growth promoters in animal diets and their health effects on consumers of poultry products has made it necessary to search for suitable alternative growth-promoting agents, in particular medicinal plants. Aim of this study was to determine the effects of dietary supplementation of *Vernonia amygdalina* leaf meal (VALM) on haematological and biochemical parameters and growth performance of broilers chickens. Four hundred and eighty (480) day-old Cobb chicks were randomly allocated to four dietary treatment diets: a basal diet (V0) (control group with 0% of VALM); basal diets with 1 (V1), 2 (V2) and 3% VALM (V3) having 4 replicates of 30 birds each in a completely randomised design. Birds were managed conventionally on a deep litter system for a period of 6 weeks. Data on body weight, feed intake, feed conversion ratio and blood parameters were collected. In birds of the V2 and V3 groups feed intake was significantly lower ($P = 0.012$) than in group V0. In chickens of the groups V0 and V1 the feed conversion ratio was similar but significantly higher than in groups V2 and V3. Final body weights and weight gain were significantly ($P = 0.008$) higher in the birds of the V2 group compared with chickens of the other treatment groups. Also, the weights in groups V0 and V1 were lower than in group V3. Except for packed cell volume and white blood cells, the results showed no differences between the groups in the other haematological parameters measured. Total cholesterol and low-density lipoprotein cholesterol concentration were lower in the birds offered VALM than in the control group. However, there was no difference in total protein but the values of albumin were higher ($P = 0.002$) with VALM application, especially in birds of the V2 group. It can be concluded that supplementation with VALM at a dose of 2% improved growth performance and haematological and biochemical parameters in broilers.

Key words

Vernonia amygdalina; leaf meal; body weight; feed conversion ratio; blood parameter; blood serum; biochemical indices

Zusammenfassung

Wachsende Bedenken gegenüber dem Einsatz von Antibiotika als Wachstumsförderer in der Tierernährung und ihre gesundheitlichen Auswirkungen auf die Verbraucher von Geflügelprodukten machten es notwendig nach geeigneten alternativen wachstumsfördernden Mitteln, insbesondere Heilpflanzen, zu suchen. Ziel dieser Studie war es, die Auswirkungen einer Nahrungsergänzung mit *Vernonia amygdalina* – Blattmehl (VALM) auf hämatologische und biochemische Parameter und die Wachstumsleistung von Masthühnern zu untersuchen. Vierhundertachtzig (480) Eintagsküken der Linie Cobb wurden nach dem Zufallsprinzip auf vier Fütterungsgruppen verteilt: eine Kontrollgruppe (V0), die eine Grundfuttermischung ohne (0%) VALM-Zusatz erhielt; drei Versuchsgruppen, an die die gleiche Grundfuttermischung aber mit unterschiedlichen Anteilen an VALM (1%: V1, 2%: V2 und 3%: V3) verfüttert wurde. Insgesamt erfolgten je Versuchsgruppe 4 Wiederholungen mit jeweils 30 Tieren in einem vollständig randomisierten Design. Die Tiere wurden konventionell auf Einstreu über einen Zeitraum von 6 Wochen gehalten. Körpergewicht, Futteraufnahme, Futterverwertung und Blutparameter wurden erfasst. Verglichen mit der Gruppe V0 wiesen die Tiere in den Gruppen V2 und V3 eine signifikant niedriger ($P = 0,012$) Futteraufnahme auf. Bei Hühnern der Gruppen V0 und V1 war die Futterverwertung ähnlich, aber signifikant höher als in den Gruppen V2 und V3. Das Endkörpergewicht und die Gewichtszunahme erreichten bei den Hühnern der Gruppe V2 im Vergleich zu den Hühnern der anderen Behandlungsgruppen signifikant ($P = 0,008$) höhere Werte. Die Gewichte in den Gruppen V0 und V1 waren niedriger als in der Gruppe V3. Mit Ausnahme des Hämatokrits und der weißen Blutkörperchen zeigten die übrigen gemessenen hämatologischen Parameter keine Variationen zwischen den Gruppen. Die Konzentrationen an Gesamt-Cholesterin und Lipoprotein-Cholesterin niedriger Dichte waren bei Hühnern, denen VALM angeboten wurde, niedriger als in der Kontrollgruppe. Beim Gesamtprotein gab es keinen Unterschied zwischen den Gruppen, aber die Albuminwerte waren bei VALM-Zusatz zum Futter signifikant höher ($P = 0,002$), insbesondere in der V2-Gruppe. Die Schlussfolgerung aus den Untersuchungen ist, dass durch eine Futtermittelergänzung mit 2% VALM die Wachstumsleistung sowie die hämatologischen und biochemischen Parameter bei Masthähnchen verbessert werden können.

Stichworte

Vernonia amygdalina; Blattmehl; Körpergewicht; Futterverwertung; Blutparameter; biochemische Indizes; Bluserum

Introduction

Feed additives are ingredients added to poultry diets to enhance production efficiency, improve health and reduce morbidity. The use of antibiotics as growth promoters to improve the production and profitability in poultry has been banned (FALLAH et al., 2013). It is therefore necessary to find alternatives to improve profitability of poultry production (ATTIA and AL-HARTHI, 2015; ATTIA et al., 2017; ATTIA et al., 2018). Phytobiotics are a suitable alternative that has attracted attention in recent years. Phytobiotics are generally defined as parts of plants or extracts thereof which are incorporated into animal feed to improve their productivity and the quality of the food obtained from these animals (WINDISCH et al., 2008). The nutrient content of leaves varies considerably from one plant to another and is a good source of vitamins, essential amino acids, proteins and minerals (AL-HARTHI et al., 2009; FASUYI, 2006). Plants also contain active components such as flavonoids, glycosides, saponins and tannins, which possess medicinal properties (DOUGHARI et al., 2009). Although phytochemicals have many useful effects on animal health, several reports have shown that some of them are potentially toxic and can cause poisoning (KHATTAB et al., 2010; LORENT et al., 2014). Generally, blood components (biochemical and haematological) are influenced by the quantity and quality of food and serve as a biomarker of the status of animals exposed to toxic substances and other pathological conditions (OLAFEDEHAN et al., 2010). The haematopoietic system is considered an important blood parameter of physiological and pathological status in animals since it is one of the most sensitive targets for toxic agents (BABATUNDE et al., 1992). For a feedstuff to be considered safe for animal health, its effect on blood parameters must be studied in order to understand the nutritional potential and safety based on their acceptability (ATTIA et al., 2017). *Vernonia amygdalina* is a good source of phytonutrients such as proteins, carbohydrates, minerals and vitamins (USUNOMENA and NGOZI, 2016). It also contains other components such as tannins, steroids, terpenoids, flavonoids, glycosides, saponins, alkaloids and polyphenols (OLASUPO et al., 2017). *Vernonia amygdalina* is a small tree and one of the edible vegetables in Togo and other parts of the African sub regions (IGBAKIN and OLOYEDE, 2009). It is used for both medicinal and nutritive purposes (ADARAMOYE et al., 2008). It is popularly called bitter leaf because of its abundant bitter component (EKPO et al., 2007). It is one of the natural feed additives, which might be of great productive and health importance in the broiler industry. To our knowledge, there is a scarcity of scientific reports on the evaluation of *Vernonia amygdalina* leaf meal (VALM) on growth performance and physiological parameters of broiler chickens when given as feed supplement. Therefore, the present study was designed to evaluate the efficacy of VALM supplementation on growth performance and haematological- and biochemical parameters in broilers.

List of Abbreviations

ANOVA:	Analysis of Variance
BW:	Body Weight
CERSA:	Centre d'Excellence Régional sur les Sciences Aviaires
CP:	Crude Protein
CVD:	Cardiovascular Disease
FCR:	Feed Conversion Ratio
FI:	Feed intake
Hb:	Haemoglobin
HDL:	High-Density Lipoprotein
HSD:	Honestly Significant Difference
LDL:	Low Density Lipoprotein
MCH:	Mean Corpuscular Haemoglobin
MCHC:	Mean Corpuscular Haemoglobin Concentration
MCV:	Mean Corpuscular Volume
ME:	Metabolizable Energy
PCV:	Packed Cell Volume
RBC:	Red Blood Cell
RH:	Relative Humidity
VALM:	*Vernonia amygdalina* Leaf Meal
VLDL:	Very Low-Density Lipoprotein
WBC:	White Blood Cell

Material and methods

This study was carried out in accordance with the regulations on animal welfare in Togo and the ethics committee of the research centre approved all the procedures involving the handling of animals.

Experimental site

The experiment was carried out at the poultry experimental unit of Centre d'Excellence Régional sur les Sciences Aviaires (CERSA) of University of Lomé, Togo. The experiment was conducted for 6 weeks. The birds were housed in an open-sided deep litter house with natural environment, ventilation, temperature and humidity. The average ambient temperature and relative humidity (RH) were 32 ± 5°C and 45 ± 4%, respectively. The lighting regimen was 23:1 light-dark cycle. The litter materials (wood shavings) were changed every week.

Preparation of plant leaf powder

Fresh *Vernonia amygdalina* was harvested at Adéticopé, a village located 30 km north of Lomé. It was dried in a fresh room at 18°C and pulverised into powder.

Experimental design and management

A total number of 480 day-old male broiler chicks of the Cobb strain were used in a completely randomised design. The birds were assigned to four treatments with 120 birds each, having 4 replicates of 30 birds each with a stocking density of 30 kg/m². The birds were vaccinated against Gumboro disease (on day 4) and against Newcastle disease and Infectious bronchitis (on day 5). A booster of these three vaccines was given after two weeks. The vaccines were administered orally.

Four diets were formulated for each of the starter and finisher phases of the experiments. The treatments were: basal diet supplemented with 0 (V0), 1 (V1), 2 (V2) and 3% (V3) VALM. Each diet was formulated to fit crude protein (CP) and metabolizable energy (ME). The composition of the starter and finisher diets is shown in Table 1. The birds were

fed starter mash experimental diets for the first 4 weeks. At the 5th week of age, the birds were placed on finisher mash experimental diets. Feed and water were provided *ad libitum*.

Table 1. Ingredients and chemical composition of the experimental diets

Inhaltsstoffe und chemische Zusammensetzung des Versuchsfutters

	Starter 0–4 weeks				Finisher 5–6 weeks			
Ingredients	**V0**	**V1**	**V2**	**V3**	**V0**	**V1**	**V2**	**V3**
White maize	57.00	57.00	57.00	56.90	66.00	66.30	66.70	66.30
Roasted soya bean meal	25.00	24.50	25.00	25.00	24.00	23.50	22.5	23.20
Wheat bran	5.00	4.50	4.00	3.50	3.00	2.20	1.00	1.00
Fish meal 40%	5.00	5.00	4.00	4.10	2.00	2.00	3.30	2.30
Oyster shell	2.00	2.00	2.00	2.00	2.50	2.50	2.00	1.70
VALM	0.00	1.00	2.00	3.00	0.00	1.00	2.00	3.00
Concentrate	5.00	5.00	5.00	4.50	2.50	2.50	2.50	2.50
Lysine	0.50	0.50	0.50	0.50	–	–	–	–
Methionine	0.50	0.50	0.50	0.50	–	–	–	–
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values								
Metabolizable energy (MJ)	12.7	12.7	12.7	12.7	13.0	13.0	13.0	13.0
Crude protein (%)	21.43	21.41	21.42	21.44	18.45	18.40	18.42	18.41
Crude fibre (%)	4.71	4.72	4.77	4.79	4.74	4.72	4.67	4.75
Lysine (%)	1.37	1.36	1.33	1.31	1.14	1.15	1.12	1.14
Methionine (%)	0.54	0.52	0.60	0.56	0.43	0.46	0.44	0.44
Methionine + Cystine (%)	0.68	0.65	0.67	0.69	0.54	0.56	0.51	0.52
Calcium (%)	1.17	1.18	1.15	1.15	1.13	1.14	1.18	1.14
Phosphorus (%)	0.66	0.66	0.64	0.62	0.50	0.49	0.49	0.47

VALM: *Vernonia amygdalina* leaf meal
V0-V3: experimental groups with different levels of VALM in the diet; V0 (0%), V1 (1%), V2 (2%), V3 (3%)

Growth performance

At the onset of the experiment, initial body weights of the birds were recorded. Feed intake (FI) and body weight (BW) were measured weekly. Mortality was recorded as it occurred. Weight gain, average daily weight gain, FI, average daily FI and feed conversion ratio were calculated. The weight gain was calculated as the difference between the final weights and the initial weights. FI was determined as the difference between the amount of feed offered and refusals. Feed conversion ratio (kg feed/kg gain) was calculated by dividing FI by BW gain (MWALE et al., 2008).

Haematological and biochemical parameters

Blood collection was carried out at the end of the experiment (6th week). Five (5) birds were selected at random from each of the treatments and bled via the wing vein using sterile needles and syringes. Two (2.0) ml of blood was collected into each sterilised bottles containing EDTA as anticoagulant for haematological analysis. The packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), and haemoglobin (Hb) concentrations were measured using Mindray BC 2800, an automated system for the determination of haematological parameters. Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) levels were calculated from PCV, Hb, and RBC according to BUSH (1991). Another 2.0 ml of blood was collected from the birds without anticoagulant into a vacutainer to determine serum biochemical indices (total protein, albumin, triglycerides, cholesterol, Low Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL) level in the blood) by using the colorimetric method from the specific reagent kits with BIOBASE BK-F96PRO spectrophotometer. Globulin value was calculated as the difference between total protein and total albumin. The plasma Very Low Density Lipoprotein (VLDL) was estimated using the formula of FRIEDEWALD et al. (1972):

$$VLDL = \frac{PlasmaTriglycerides}{5}$$

Data analysis

All results are expressed as means ± standard error of the mean (SEMs). The effects of dietary VALM supplementation dose were assessed using linear regression and ANOVA one-way analysis. The linear model was as follows:

$Y_{ij} = \mu + \beta_1 + eij$

Y_{ij} = dependent variable, μ = overall mean, β_1= effect of VALM

When significant, Tukey's Honestly Significant Difference (HSD) was used to separate the means. $P < 0.05$ was considered significant. All statistical analyses were performed using the GraphPad Prism 7.00 package.

Results

Body weight, feed intake and feed conversion ratio

The results of VALM on growth performance are presented in Table 2. There was a significant difference ($P = 0.024$) in daily FI of the birds. FI of the birds in V3 and V2 was similar but significantly lower ($P = 0.012$) than those of V1 and V0. Also, FI in group V1 was lower than that of group V0. The final BW of birds in V2 was higher than that of V3, whose value was higher than that of V0 and V1. There was a similar final weight of the birds in V0 and V1. A similar trend was observed in the weight gain of the birds. The feed conversion ratio of the birds in V0 and V1 was similar and higher ($P = 0.001$) than those of V2 and V3, whose values were comparable and better than that in the other groups.

Table 2. Effect of different levels of dietary *Vernonia amygdalina* leaf meal (VALM) on body weight gain, feed intake and feed conversion ratio

Einfluss unterschiedlicher Anteile an *Vernonia amygdalina* Blattmehl (VALM) im Futter auf Körpergewichtszunahme, Futteraufnahme und Futterverwertung

Parameters	Treatments				SEM	*P*-value
	V0	V1	V2	V3		
Initial body weight (g)	43.2	42.7	43.1	43.5	0.10	0.120
Final body weight (g)	1725^{c}	1726^{c}	1765^{a}	1758^{b}	7.73	0.008
Weight gain (g)	1682^{c}	1684^{c}	1722^{a}	1714^{b}	7.19	0.002
Daily weight gain (g)	40.0^{c}	40.0^{c}	41.0^{a}	40.8^{b}	0.17	0.038
Total feed intake (g)	3602^{a}	3557^{b}	3485^{c}	3424^{c}	6.48	0.012
Daily feed intake (g)	85.7^{a}	84.6^{b}	82.9^{c}	83.7^{c}	0.42	0.024
Feed conversion ratio (kg feed/kg gain)	2.14^{a}	2.11^{a}	2.02^{b}	2.05^{b}	0.01	0.001

^{abc} Means with different superscripts on the same row differ significantly ($P < 0.05$)
V0-V3: experimental groups with different levels of VALM in the diet; V0 (0%), V1 (1%), V2 (2%), V3 (3%)

Haematological variables

Table 3 shows the haematological parameters of the various treatments. Hb and RBC concentrations of the birds across the treatment groups were not significantly different ($P = 0.183$). PCV of the birds fed VALM were similar and higher ($P = 0.021$) than those of the birds in the control groups. The PCV values were within the normal range of 25% – 45%. However, the total WBC count of the birds in the V0 group was significantly lower than those of the birds fed VALM.

Table 3. Effect of different level of dietary *Vernonia amygdalina* leaf meal on blood parameters of broiler chickens

Einfluss unterschiedlicher Anteile an *Vernonia amygdalina* Blattmehl (VALM) im Futter auf Blutparameter

Parameters	Treatments				SEM	*P*-value
	V0	V1	V2	V3		
PCV (%)	32.9[b]	38.3[a]	39.0[a]	38.1[a]	1.35	0.021
RBC ($\times 10^{12}$ /l)	2.59	2.61	2.64	2.60	0.07	0.153
WBC ($\times 10^{9}$ /l)	185[b]	198[a]	197[a]	194[a]	1.46	0.039
Hb (g/dl)	11.3	11.4	11.9	11.4	0.20	0.214
MCV (fl)	127	131	129	133	2.30	0.198
MCHC (g/dl)	36.2	37.3	36.8	37.6	2.58	0.225
MCH (pg)	45.9	46.9	46.1	46.6	2.50	0.524

[ab] Means with different superscripts on the same row differ significantly (P < 0.05). PCV = packed cell volume, RBC = red blood cells, WBC = white blood cells, Hb = haemoglobin, MCV = mean cell volume, MCHC = mean cell haemoglobin concentration, MCH = mean cell haemoglobin.
V0-V3: experimental groups with different levels of VALM in the diet; V0 (0%), V1 (1%), V2 (2%), V3 (3%)

Biochemical variable

Results in Table 4 reveal that VALM significantly reduced (P = 0.001) serum cholesterol in the blood plasma. The cholesterol of the birds in V0 was higher than those of the other treatment groups, while the level in the birds in V1 was higher than those of V2 and V3 whose values were comparable. A similar trend was observed in the LDL and triglyceride of the birds. The birds fed VALM had a similar HDL which was higher than those of the birds in the control group. VLDL values were significantly lower (P = 0.003) in the treated groups compared to the control group. The total protein of the birds in V0, V1 and V3 was similar and lower than that of V2. The albumin of the birds in V0 was lower than those of the other treatment groups, while the values of the birds of V1 and V3 was similar but lower (P = 0.02) than that of V2.

Table 4. Effect of different level of dietary *Vernonia amygdalina* leaf meal on biochemical parameters of broilers chicken

Einfluss unterschiedlicher Anteile an *Vernonia amygdalina* Blattmehl (VALM) im Futter auf biochemische Parameter

Parameters	Treatments				SEM	*P*-value
	V0	V1	V2	V3		
Cholesterol (mg/dl)	139.3[a]	118.7[b]	108.2[c]	107.3[c]	2.48	0.0001
LDL (mg/dl)	122.3[a]	113.7[b]	93.3[c]	93.8[c]	3.39	0.0036
HDL (mg/dl)	36.4[b]	74.1[a]	75.1[a]	75.2[a]	2.40	0.0012
VLDL (mg/dl)	9.2[a]	7.4[b]	5.5[c]	5.1[c]	1.40	0.0019
Triglycerides (mg/dl)	46.3[a]	37.0[b]	27.8[c]	25.2[c]	2.18	0.0082
Total protein (g/l)	38.6[b]	40.8[b]	45.6[a]	41.9[b]	1.69	0.0234
Albumin (g/l)	21.7[c]	22.2[b]	24.3[a]	23.0[b]	0.33	0.0020
Globulin (g/l)	16.8	16.8	18.2	18.2	1.83	0.9071

[abc] Means with different superscripts on the same row differ significantly (P < 0.05). LDL = low density lipoprotein, HDL = high density lipoprotein, VLDL = very low density lipoprotein.
V0-V3: experimental groups with different levels of VALM in the diet; V0 (0%), V1 (1%), V2 (2%), V3 (3%)

Discussion

Body weight, feed intake and feed conversion ratio

In this study it could be clearly shown that incorporation of VALM in the broiler diet had effects on growth performance and on haematological- and biochemical parameters. FI decreased significantly (P = 0.012) in the birds fed VALM compared to those in the control group (V0). This observation can be attributed to the bitter taste of the feed (MOHAMMED and ZAKARIYA'U, 2012). The higher concentration of antinutritional factors, such as alkaloids, saponins, tannins and glycosides, in *Vernonia amygdalina* as reported by ARHOGHRO et al. (2009) may have caused bitter taste and hindered the ingestion of the feed containing higher concentration of *Vernonia amygdalina* (BONSI et al., 1995; HINDRICHSEN, 2000). The growth performance of the birds in the present study shows that the final BW, weight gain and feed conversion ratio of the birds were significantly different. The birds fed VALM had better weight gain and feed conversion ratio. The result of the present study is in line with the findings of TANGKA (2003) and DURUNNA et al. (2011) who reported improved growth performance of birds fed bitter leaf. The improvement in weight gain observed in the treated group resulted in a lower FCR. OLOBATOKE and OLONIRUHA (2009) reported that inclusion of bitter leaf powder in cockerel diets significantly improved FCR. ADARAMOYE et al. (2008) suggested that the observed improvement may be associated with the beneficial effect of the bitter leaf to strengthen the gastrointestinal enzymes and thereby improve digestion and assimilation of nutrients. HUFFMAN (1996) also reported that bitter leaf enhanced gastrointestinal enzymes (chymotrypsin) production, which may improve not only the utilisation of feed but could aid in the digestion of sporozoites and other intestinal parasites that could cause decreased utilisation of the feed. In contrast, MOHAMMED and ZAKARIYA'U (2012) observed that inclusion of bitter leaf as a feed additive did not significantly improve weight gain and FCR in broilers. The birds fed 2% of VALM had the highest weight gain (1722 g) at the end of the experiment. This indicates an optimal level of VALM inclusion for better performance and could be recommended to farmers, although further experiments seem necessary.

Haematological variable

In the present study, there was no influence of the dietary treatment on haematological indices among the treatments, except for PCV and WBC. On the other side, there was a marginal increase in RBC levels of birds fed VALM, a finding revealing that VALM may have a possible potential to stimulate erythropoietin release from the kidneys, which is the humoral regulator of RBC production. OSHO et al. (2014) demonstrated that oral administration of bitter leaf extract on broiler chickens did not have significant effects on the Hb and RBC in treated birds. According to MAXWELL et al. (1990), blood parameters are important in assessing the quality and suitability of feed ingredients for farm animals. It's also reported that blood parameters are the major indices of the physiological, pathological, and, when compared to normal values, nutritional status of an organism. Changes in the constituent compounds of blood could be used to interpret the metabolic stage of an animal as well as the quality of the feed (ATTIA et al., 2017; ATTIA et al., 2018; ATTIA et al., 2019; BABATUNDE et al., 1992). The higher PCV and WBCs in birds offered VALM are in agreement with earlier findings of OWEN and AMAKIRI (2011) who showed that, with the exception of WBC, all other haematological indices measured were influenced by increasing levels of VALM. The numerical differences observed in the PCV, Hb, RBC, and WBC of birds fed VALM suggest that the feed were better utilised and assimilated into the blood stream of the birds (ONU, 2010). The result of this study shows that VALM can alter the distribution and occurrence of WBC, suggesting the ability of *Vernonia amygdalina* to act as an immune stimulant. The haemoglobin level and PCV was higher in the birds of the V2 group than in the other treatments. Acute inflammation from most pathogenic micro-organisms results in haemolysis, which is manifested in a lower level and PCV (KUMARNSIT et al., 2006). The higher values of these haematological indices in the birds administered VALM may be due to their inability to cause haemolysis resulting from the anti-inflammatory potentials inherent in *Vernonia amygdalina*. There was no significant difference in MCHC, MCH and MCV in the groups receiving VALM compared to the control group. However, there was a slight increase in the values. This slight increase indicates that there were differences in corpuscle sizes, even if the blood samples had similar levels. MCHC, MCH and MCV measurements are used to diagnose anaemia and as an index of the bone marrow's ability to form RBC (ALETOR and EGBERONGBE, 1992). Therefore, the normal range of the values obtained in the present study indicates that the birds were not anaemic. Variations in avian haematological blood values are a normal phenomenon, and depend in most cases on the physiological condition of the birds (ATTIA and AL-HARTHI, 2015; ISLAM et al., 2004).

Biochemical variables

A lipid profile (which involves levels of total cholesterol, HDL, LDL, VLDL and triglycerides) serve as diagnostic indices in conditions such as chronic obstructive jaundice, hepatitis, coronary heart disease and atherosclerosis. Hyperlipidaemia is one of the risk factors for coronary heart disease while cholesterol is the major lipid constituent of atherosclerotic plaque (EKPO et al., 2007). In this study, addition of *Vernonia amygdalina* to the diets significantly reduced cholesterol and triglyceride concentrations of the broiler chickens, indicating that the plant could contribute to a decrease of diseases associated with hyperlipidaemia (ATTIA et al., 2017; ATTIA et al., 2018; ATTIA et al., 2019). The elevated levels of triglyceride and cholesterol obtained in the broiler chickens in the control group (without VALM) are in agreement with the findings of EKPO et al. (2007). The results suggest that VALM reduces triglyceride biosynthesis and promotes the redistribution of cholesterol between lipoprotein molecules. This observation is consistent with the findings of OJIAKO and NWANJO (2009) in which *Vernonia amygdalina* significantly attenuated the hepatic triglyceride and LDL cholesterol levels of streptozotocin diabetic rats. Similar results were reported by AL-HARTHI et al. (2009). OWEN and AMAKIRI (2011) also reported serum cholesterol and LDL lowering potentials of bitter leaf meal. Increased activity of the enzyme catalase involved in esterification of cholesterol in the plasma could have influenced the lower serum cholesterol in the treated groups. From this study, incorporation of VALM into finisher broiler diets numerically elevated HDL-cholesterol, indicating its promising protective role against cardiovascular disease (CVD). The protective roles of HDL cholesterol from CVD have been suggested to occur in various ways (NOFER et al., 2002). HDL exerts part of its anti-atherogenic effect by counteracting LDL oxidation. Recent studies also showed that HDL promotes the reverse cholesterol transport pathway by inducing an efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL (YOKOZAWA et al., 2006). Our study shows that probably already 2% *Vernonia amygdalina* have an anti-atherogenic effect by increasing HDL cholesterol. LDL-cholesterol is another primary target of CVD risk-reduction therapy (KWITEROVICH, 1997). In the present study, *Vernonia amygdalina* elicited beneficial effects by lowering the levels of cholesterol including LDL of the broiler chickens. Total protein and albumin tests are usually used to evaluate the health status of animals. These biomarkers are often used in diagnosing diseases and in monitoring changes in health status of farm animals. The total protein is composed of albumin and globulin in the blood and reflects the nutritional status of the birds. Low levels of albumin indicate incidence of disease related to liver or kidney. It could also be associated with the presence of infection. Total protein of the birds in the V2 group was higher than those of the other treatments, indicating an optimum level. A similar trend was observed for albumin. This finding confirms an earlier report by OWEN and AMAKIRI (2011).

Conclusion

Considering the effects of different levels of *Vernonia amygdalina* leaf meal on weight gain, FI and feed gain ratio of the broiler chickens, it appears that VALM at the level of 2% is optimum for improvement of growth performance and physiological responses in broiler chickens. It should be recommended for poultry production, although further experiments are recommended.

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Authors´ Contribution

Conceptualization: B.M. Tokofai, K. Idoh, O. E. Oke and A. Agbonon; methodology: B.M. Tokofai, K. Idoh, O. E. Oke and A. Agbonon; experimental works: B.M. Tokofai, K. Idoh; statistical analysis: B.M. Tokofai and K. Idoh; data validation: A. Agbonon; draft preparation: B.M. Tokofai, K. Idoh and A. Agbonon; writing, review and editing: B.M. Tokofai, K. Idoh, O. E. Oke and A. Agbonon; supervision: A. Agbonon; funding acquisition: A. Agbonon

All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest

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