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HAEMATOLOGICAL AND SERUM BIOCHEMICAL RESPONSE OF BROILER CHICKENS TO VARIED CONCENTRATIONS OF BITTER LEAF (*Vernonla anygdalina*) EXTRACT IN DRINKING WATER

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ABSTRACT

The study investigated the effects of bitter leaf (*Vernonia amygdalina*) extract in drinking water on the blood profiles of broiler chickens. A total of 150-day-old broiler chickens were randomly allocated to five treatments of bitter leaf extract, viz; 0, 30, 60, 90, and 120 ml per litre of drinking water. There were thirty birds per treatment, and each treatment had three replicates. Data were collected on proximate composition of bitter leaf meal, commercial feeds fed and haematological and serum indices. Results indicated significant differences ($p<0.05$) in haematological parameters across treatments, with higher PCV of 28.33% observed in 90 ml/L. Haemoglobin was higher ($p<0.05$) in birds given 90 ml/L (9.67g/dl). RBC count ranged from 2.22 to 2.48 $x10^{12}$/L, with the highest count observed in 120 ml/L. MCV values ranged from 39.17 to 39.29pg, with 30 ml/L showing the highest value. MCH and MCHC were highest in 30 ml/L at 39.29pg and 33.41%, respectively. WBC count ranged from 209.00 to 224.43 $x10^{9}$/L across treatments. No significant differences ($p>0.05$) were found in total protein, albumin, and globulin levels across treatments. However, AST and ALT levels showed increasing trends with higher concentrations of bitter leaf extract, reaching 59.00µ/dl and 10.50µ/dl, respectively, in birds on 120 ml/L. It was concluded that, bitter leaf extract influenced haematological parameters positively. However, 90ml/L is recommended for broiler chickens for optimum benefits.

Keywords: *Vernonia amygdalina*, Haematology, Serum, Extract, Broiler Chicken

INTRODUCTION

Phytogenic feed additives, for example, essential oils, are known for their antimicrobial, antioxidant, and anti-inflammatory effects (Windisch *et al.*, 2008). Herbs and spices also contain phytochemicals that may positively influence broiler performance and overall well-being (Hashemipour *et al.*, 2013). It has been reported that phytogenic feed additives possess potential to improve animal growth performance, feed efficiency, and immune function without the drawbacks associated with certain synthetic additives (Windisch *et al.*, 2008; Hashemipour *et al.*, 2013). Moreover, the use of phytogenic feed additives is consistent with increasing consumer demand for products derived from animals raised with more natural and sustainable practices (Windisch *et al.*, 2008).

In response to these challenges, feed additives, particularly herbal plants like bitter leaf (*Vernonia amygdalina*), have emerged as potential solutions. The reported success of bitter leaf in replacing portions of conventional diets, coupled with its rich phytochemical composition, positions it as a promising candidate to positively influence broiler growth and blood parameters, (Owen and Amakiri, 2011).

Vernonia amygdalina leaf meal (VALM) has been reported to contain 527.83kcal/kg metabolizable energy, 86.40% dry matter, 21.50% crude protein, 13.10% crude fibre, 6.80% ether extract, 11.05% ash. Mineral profile indicates that *V. amygdalina* has 3.85% calcium, 0.40% magnesium, 0.03% phosphorus, 0.006% iron, 0.33% potassium and 0.05% sodium (Owen and Amakiri, 2011). Ojiako and Nwanjo (2016) reported that the administration of bitter leaf significantly reduced the low density lipoprotein (LDL) cholesterol level of broiler chickens. Osinubi (2006) reported that the increased activity of the enzyme catalase involved in esterification of cholesterol in the plasma could have influenced the decrease in serum cholesterol in the bitter leaf extract administered birds. Birds on bitter leaf aqueous extract had better high density lipoprotein (HDL) values compared to those on the control. Chiemela and Nwakpu (2016) reported improved haemoglobin level and packed cell volume with 1.00% of the bitter leaf meal as compared to the control. However, Bonsi *et al.* (1995) reported improvement in the lymphocyte count in the sheep that were treated with the bitter leaf meal. This research therefore sought to determine the haematological and serum biochemical response of broiler chickens to varied concentrations of bitter leaf (*Vernonla anygdalina*) extract in drinking water.

MATERIALS AND METHODS

Experimental Location

The study was conducted within the premises of Prince Abubakar Audu University, Anyigba. The Poultry Unit of the Livestock Teaching and Research Farm of the Department of Animal Production was used for the experiment. Anyigba, which is in Dekina Local Government Area of Kogi State falls within Latitude: 7.4934° N and Longitude: 7.1628° E of the Greenwich meridian (Google Maps. 2024).

Collection, Processing and Preparation of the Bitter leaf and its Extract

Fresh bitter leaves (*Vernonia amygdalina*) were collected from within Prince Abubakar Audu University Campus. The

leaves were sundried and thereafter ground into powder in an attrition mill at Anyigba market. The resultant powder was then packed into polyethylene bag and preserved in the feed storage room until required. One hundred grams (100g) of the ground *Vernonia amygdalina* leaf powder was weighed and soaked in 1litre of hot water (at 100^0C) for 24 hrs. It was then decanted and sieved white using muslin cloth to obtain the *Vernonia amygdalina* leaf extract. Graded levels of the extract i.e. 0, 30, 60, 90 and 120ml were added to 1 litre of drinking water, each in treatments 1, 2, 3, 4 and 5, respectively. This procedure was carried out daily. A common commercial diet procured within Anyigba was fed to the birds at both starter and finisher phases.

Experimental Layout and Management of Birds

One hundred and fifty (150) day old broiler chickens of Arbor Acre Strain were used for the experiment. The birds were weighed on arrival and randomly allocated to the five treatments in a Completely Randomized Design (CRD). Each treatment, split into three replicates, had thirty birds (30), such that each replicate had ten (10) birds. The poultry house was a deep litter system. The experiment lasted for seven weeks. Feed and drinking water containing bitter leaf extract were provided *ad libitum* and standard routine management practices were followed.

Chemical Analysis

Bitter leaf was evaluated both for some phytochemical contents and its proximate composition. Proximate composition of the commercial feeds was also analyzed (Harbone, 1973; Sofowora, 1980; AOAC, 2005) and is as shown in Table 1.

Table 1: Proximate Composition of Experimental Diets of Broiler Chickens

Parameter	Starter	Finisher
Crude protein (%)	21.00	17.00
Crude fat (%)	4.00	4.00
Crude fibre (%)	5.00	5.00
Calcium (%)	1.00	0.85
Phosphorus (%)	0.50	0.42
Lysine (%)	1.15	0.90
Methionine (%)	0.50	0.40
Metabolizable energy kcal/kg	2900	3000

Collection of Blood Samples for Haematological Evaluation

At the end of the trial, two (2) birds per replicate were randomly selected for blood collection. Blood samples were collected through the wing vein, after disinfecting the site with methylated spirit, into EDTA bottles with the aid of sterilized syringe and needle. The haematological parameters evaluated were red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and white blood cells (WBC), neutrophil and lymphocytes, using the method reported by Ebegbulem (2018).

Collection of Blood Samples for Serum Evaluation

At the end of the experiment, blood samples were collected from two birds per replicate via wing vein puncture using 5ml syringes. The blood were poured into plain blood sample bottles (EDTA free) to allow the blood coagulate. Blood samples were analyzed for serum parameters like total protein (TP), albumin, globulin, aspartate amino-transaminase (AST), and alanine amino-transaminase (ALT) according to the methods described by Ebegbulem (2018).

Statistical Analysis

All data collected were analyzed statistically using the One-way Analysis of Variance (ANOVA) with the aid of SPSS version 20. Where there was significant difference among means, such were further separated using the same SPSS version 20 software.

RESULTS AND DISCUSSION

Results

Phytochemicals of bitter leaf meal

The phytochemicals of bitter leaf meal are shown in Table 2. Obtained values showed bitter leaf contained tannin 14.07(g/kg), phenol 12.44(g/kg), flavonoid 84.17(g/kg), alkaloid 3.15(g/kg), cardiac glycoside 22.85(g/kg) and saponin 13.02(g/kg).

Table 2: Phytochemicals of Bitter leaf meal

Parameter	
Tannin (g/kg)	14.07
Total phenol (g/kg)	12.44
Flavonoid (g/kg)	84.17
Alkaloid (g/kg)	3.15
Cardiac glycoside (µg/kg)	22.85
Saponin (g/kg)	13.02

Haematological parameters of broiler chickens offered bitter leaf extract in drinking water

The effect bitter leaf extract in drinking water on the haematology of broiler chickens is presented in Table 2. Observed results showed significant ($p<0.05$) differences in all the parameters evaluated. Values obtained for packed cell volume were, 27.00, 26.43, 27.83, 28.33 and 26.60% for treatments 0, 30, 60, 90 and 120ml/L respectively. PCV was significantly ($p<0.05$) higher with birds offered 90ml/L bitter leaf extract than other treatments. The least value was obtained with birds on 30ml/L. Haemoglobin followed similar trend as PCV. Haemoglobin values ranged from 8.80 to 9.67g/dl obtained for birds offered 30 and 90ml/L bitter leaf extract in drinking water. Red blood cell values obtained were 2.43, 2.22, 2.46, 2.46 and 2.48 $x10^{12}$/l for 0, 30, 60, 90 and 120ml/L respectively. RBC was lowest in birds on 30ml/L extract, while other treatments had similar values. Values obtained for white blood cell ranged between 209.00 and 224.43 $x10^{9}$/l obtained for the birds offered 60 and 120ml/L respectively. All the treatments had similar WBC values except at 60ml/L. Mean corpuscular volume had values ranging from 108.20 and 119.71fl. Observed values were higher in 30ml/L and 90ml/L but least in 120ml/L. While values obtained for MCH ranged from 35.80 to 39.29pg obtained for birds offered 120 and 30ml/L respectively. MCH followed similar trend as MCV. MCHC values were 33.10, 33.41, 33.43, 33.30 and 33.30% for birds offered 0, 30, 60, 90 and 120ml/L respectively, for bitter leaf extract. MCHC values were higher in the bitter leaf extract groups than the control. Neutrophil values ranged from 3.00 to 5.50% obtained for birds offered control and 60ml/L respectively. Observed values were higher in the bitter leaf extract groups than the control. Lymphocyte values ranged from 94.80 to 97.00 obtained in the birds offered 60ml/L and control. The magnitude and trend of values is in the reverse order as those for heterophils.

Table 3: Haematological indices of broiler chickens offered varied concentrations of bitter leaf extract

	Bitter leaf extract						
Parameters	0ml/L	30ml/L	60ml/L	90ml/L	120ml/L	SEM	LOS
Packed cell volume (%)	27.00^{bc}	26.43^{c}	27.83^{b}	28.33^{a}	26.60^{c}	0.38	*
Haemoglobin (g/dl)	9.00^{b}	8.80^{c}	9.28^{b}	9.67^{a}	8.86^{c}	0.13	*
Red blood cell ($x10^{12}$/l)	2.43^{a}	2.22^{b}	2.46^{a}	2.46^{a}	2.48^{a}	0.03	*
White blood cell ($x10^{9}$/l)	218.20^{a}	217.33^{a}	209.00^{b}	222.50^{a}	224.43^{a}	2.16	*
Mean corpuscular volume (fl)	112.00^{b}	119.71^{a}	113.67^{b}	117.83^{a}	108.20^{c}	1.67	*
Mean corpuscular haemoglobin (pg)	37.50^{b}	39.29^{a}	37.83^{b}	39.17^{a}	35.80^{c}	0.54	*
Mean corpuscular haemoglobin concentration (%)	33.10^{b}	33.41^{a}	33.43^{a}	33.30^{a}	33.30^{a}	0.07	*
Heterophil (%)	3.00^{c}	4.14^{b}	5.50^{a}	4.67^{b}	5.20^{a}	0.29	*
Lymphocyte (%)	97.00^{a}	95.86^{b}	94.50^{c}	95.33^{b}	94.80^{c}	0.29	*

^{abcd} Means with different superscripts along the same row show significant difference at $p<0.05$, * = significantly different, SEM= Standard Error of the Mean. LOS= level of significance

Serum biochemistry of broiler chickens offered varied inclusion levels of bitter leaf extract in drinking water

The effect of bitter leaf extract in drinking water on the serum biochemical profile of broiler chickens is presented in Table 4. There were no significant ($p>0.05$) differences in the serum total protein, albumin and globulin. However, aspartate transaminase and alanine transaminase values varied significantly ($p<0.05$). Values obtained for total protein were, 3.68, 3.67, 3.80, 3.60 and 3.63% in the birds offered 0, 30, 60, 90 and 120 ml/L respectively. Albumin values obtained ranged from 1.88 to 1.93g/dl obtained for the 120 and 60ml/L bitter leaf extract birds. Globulin values obtained were within the range of 1.68 and 1.78g/dl. Aspartate transaminase

increased significantly ($p<0.05$) from 57.33 to 59.00 µ/dl with increase in bitter leaf extract. Similarly, alanine transaminase values significantly increased from 4.67 to 10.50µ/dl with increase in the aqueous extract of bitter leaf from 0 to 120ml/L.

Table 4: Serum biochemistry of broiler chickens offered bitter leaf extract in drinking water

	Bitter leaf extract						
Parameters	0ml/L	30ml/L	60ml/L	90ml/L	120ml/L	SEM	LOS
Total protein (g/dl)	3.68	3.67	3.80	3.60	3.64	0.37	NS
Albumin (g/dl)	1.98	1.90	1.93	1.92	1.88	0.32	NS
Globulin (g/dl)	1.70	1.77	1.87	1.68	1.76	0.62	NS
Aspartate transaminase (µ/dl)	57.33^{b}	57.33^{b}	57.50^{b}	58.33^{a}	59.00^{a}	0.46	*
Alanine transaminase (µ/dl)	4.67^{d}	6.00^{c}	7.83^{b}	8.00^{b}	10.50^{a}	0.51	*

^{abcd} Means with different superscripts along the same row show significant difference at $p<0.05$, * = significantly different, SEM= Standard Error of the Mean. NS= Not significant, LOS= level of significance

Discussion

Bitter leaf contains tannins, which may inhibit digestive enzymes, thereby undermine protein digestibility and also affect nutrient absorption in birds at extreme values above 20.00g/kg ((Nworgu *et al*., 2007); consequently, reducing their growth. However, Akinola *et al*. (2018) reported that bitter leaf phenols level in moderate amount ranging from 10 to 20g/kg to be beneficial having antioxidant properties that protect poultry feed from oxidative damage, helping maintain its nutritional quality. This is within range with the 12.44g/kg obtained in this research. Phenols also offer immune regulation and disease prevention benefits, enhancing the health and productivity of poultry (Ologhobo *et al*., 2014). Esonu *et al*. (2001) reported that bitter leaf contains flavonoids, which have antioxidant, antitumor, and immune-regulating properties that improve the health of birds. Value of 84.17g/kg obtained in this research is within the range of 65.00 to 100g/kg reported as tolerable by Akinola *et al*. (2018). In poultry production, flavonoids promote growth, enhance reproduction, and improve immune function. Alkaloid values obtained in this research from bitter leaf was relatively low with a value of 3.15g/kg. Alkaloids level above 6.40 was reported to have various physiological effects such as interference with the absorption of nutrients in the gut, leading to nutrient deficiencies and poor growth on birds when consumed (Nworgu et al., 2007). Glycosides in bitter leaf might result in severe cardiovascular and gastrointestinal issues in broilers with levels beyond 1-2mg/kg (Ologhobo et al., 2014). However, with the level of 22.85µg/kg present in this research, impaired cardiac function was not observed. It could be said that the cardiac glycoside potentially supported cardiovascular health in the birds. Bitter leaf also contains moderate level of saponin with a value of 13.02 with potentials for inhibiting cholesterol thereby benefiting the broiler chickens without affecting their growth (Esonu *et al*., 2001). Akinola *et al*. (2018) reported that moderate levels of saponin (10 to 20g/kg) in broiler's diet resulted in positive effects on the chicken's health.

Haematology of broiler chickens offered varied inclusion levels of bitter leaf extract in drinking water

Haematological values showed significant ($p<0.05$) differences across all the parameters evaluated. Packed cell volume (PCV), which indicates the proportion of blood volume occupied by red blood cells, remained relatively stable across treatments, with the highest value of 28.33% observed in treatment 4 (90ml/L). This suggests that increasing bitter leaf extract in drinking water to 90ml/L enhanced the volume of red blood cells in the blood. These findings align with Owen and Amakiri (2011), who found that all haematological indices, except white blood cells, were positively influenced by increasing level of bitter leaf meal, with PCV values rising from 28.04 to 31.96%. Durunna *et al.* (2011) reported PCV values ranging from 30.11 to 34.09% in broiler chickens fed diets containing *Vernonia amygdalina* leaf meal (0 to 1.5%).

Haemoglobin levels were significantly higher in birds given 90ml/L of bitter leaf extract, with a value of 9.67mg/g. Haemoglobin concentration reflects the blood's oxygen-carrying capacity, which was best enhanced in birds on 90ml/L. Osho *et al.* (2014) observed that increased haemoglobin levels (8.94 to 11.69g/dl) when *Vernonia amygdalina* extract was administered in drinking water to broiler chickens. Owen and Amakiri (2011) observed values from 8.50 to 10.71g/dl in broilers fed bitter leaf meal.

For RBC count, values ranged from 2.22 to 2.48 $x10^{12}$/L in treatments with 30ml/L and 120ml/L correspondingly. Treatment 5 (120ml/L) had the highest RBC count, suggesting that 120ml/L significantly increased RBC production, potentially stimulating erythropoietin release from the kidneys. This observation contradicts that of Osho *et al*. (2014), who reported no significant effects on Hb and RBC in treated birds. Owen and Amakiri (2011) found RBC values ranging from 2.27 to 2.58 $x10^{12}$/L in broilers fed graded levels of bitter leaf meal which is similar to the observation of this study, while Mohammed and Zakariyau

(2012) reported higher values of 2.57 to 2.94 $x10^{12}$/L when bitter leaf was used as feed additive.

White blood cell (WBC) values was 209.00 $x10^9$/L in birds given 60ml/L, while similar values (217.33, 222.50, and 224.43 $x10^9$/L) were obtained in birds given 30, 90, and 120ml/L. WBC count indicates immune function and response to infection or inflammation. Observed values fell between 220.19 and 239.43 $x10^9$/L reported by Ojiako and Nwanjo (2016) for broilers fed *Vernonia amygdalina*. Oleforuh-Okoleh *et al*. (2015) reported WBC values of 218.94 to 227.21 $x10^9$/L for birds given *Vernonia amygdalina* infusion, whereas Durunna *et al*. (2011) reported higher values of 239.53 $x10^9$/L in broilers fed 1.50% bitter leaf meal.

The highest mean corpuscular volume (MCV) values were observed in birds fed 30 and 90ml/L, with values of 39.29 and 39.17pg, respectively. MCV measures the average volume of red blood cells. The highest MCV in 30ml/L group suggests that this concentration of bitter leaf extract may result in slightly larger red blood cells. Olobatoke and Oloniruha (2009) also reported an increase in MCV values from 33.50 to 39.80fl in cockerels fed bitter leaf. Isika *et al*. (2012) reported MCV values ranging from 31.94 to 35.93fl when bitter leaf and ginger was fed to broiler chickens.

The highest values for mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were observed in birds given 30ml/L, with values of 39.29pg and 33.41%, respectively. These metrics provide insight into the amount and concentration of haemoglobin within red blood cells. The variation in MCH and MCHC across treatments indicates that bitter leaf extract affected haemoglobin content and concentration within the red blood cell. Oleforuh-Okoleh *et al*. (2015) found the highest MCH and MCHC values of 41.21pg and 33.33% in the 150ml/L bitter leaf infused group. Isika *et al*. (2012) reported MCH values ranging from 34.90 to 37.71pg and MCHC values from 32.04 to 35.00% when bitter leaf and ginger were administered to broiler chickens.

Heterophils and lymphocyte percentages remained relatively stable across treatments. Birds given 60ml/L and 120ml/L of bitter leaf extract had significantly higher neutrophil values of 5.50% and 5.20%, respectively, while the control group had the highest lymphocyte value of 97.00%. All observed haematological parameters did not indicate any adverse effect of bitter leaf extract on the health status of broiler chickens.

Serum biochemistry of broiler chickens offered bitter leaf extract in drinking water

The use of bitter leaf extract in water did not impair blood proteins, nor any metabolic health issues. Oleforuh-Okoleh *et al*. (2015) found no significant effect on serum total protein, albumin, and globulin, with values ranging from 3.41 to 3.67g/dl, 1.56 to 1.75g/dl, and 1.39 to 1.74g/dl, respectively, in birds given bitter leaf infusion. Similarly, Mohammed and Zakariyau (2012) reported no significant difference in total protein and albumin values, which ranged from 3.01 to 3.72g/dl and 1.71 to 2.15g/dl, respectively, in broilers offered bitter leaf as a feed additive.

However, both AST and ALT values showed increasing trends with higher concentrations of bitter leaf extract. Treatment 5 (120ml/L) had the highest AST and ALT values of 59.00µ/dl and 10.50µ/dl, respectively. AST and ALT are liver enzymes, and their levels in the blood can indicate liver health and function. Elevated levels suggest liver damage or stress. These results align with Isika *et al*. (2012), who reported AST and ALT values ranging from 49.04 to 62.90µ/dl and 9.04 to 11.11µ/dl, and with Ojiako and Nwanjo (2016), who reported 52.75 to 61.05µ/dl and 9.55 to 12.04µ/dl, respectively. While serum total protein, albumin, and globulin levels remained relatively stable across treatments, indicating minimal impact on overall protein metabolism, AST and ALT levels increased with increase in bitter leaf extract in drinking water.

CONCLUSION

Bitter leaf (*Vernonia amygdalina*) presents a complex profile of phytochemicals that may exert both beneficial and potentially adverse effects on broiler chickens. The high tannin content, moderate levels of phenols and flavonoids contribute antioxidant, immune-regulating, and cardiovascular-supporting properties, may enhance overall health and productivity of poultry. This was indicated by improved blood profile of birds on bitter leaf extract. Serum biochemistry evaluation showed no adverse effects on blood protein and liver enzymes levels with bitter leaf extract.

Recommendation

Poultry producers can effectively harness bitter leaf extract as a natural and sustainable strategy to enhance broiler chicken health, aligning with consumer preferences for products raised using natural feed additives. However, the best inclusion level was 90ml/L.

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