Haematology, Serum and Organ Indices of Finisher Broiler Chickens Offered Bitter leaf (*Vernonia amygdalina*) extracts in Drinking Water

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Abstract

Antibiotics are the commonly used additives in treating different infections challenging poultry production. But the ban of antibiotics in most developed countries because of its antibacterial resistance in chickens with consequential negative effect on human health prompted the need for alternative. Exploiting the potential of herbs as phytogenics became an option. Therefore, this study was conducted to investigate the role of bitter leaf extracts on the health, organ development, hematological and serum parameters of broiler chickens. Ninety Cobb 500 day-old broiler chicks were randomly assigned to three treatments (0, 50 and 100ml per litre of drinking water) of constituted bitter leaf extracts. The treatments were replicated three (3) times with 10 birds per replicate under a completely randomized design (CRD) experiment. The study commenced at the finisher phase and lasted 4 weeks. Haematological results showed that birds given 50ml/L of bitter leaf extracts had the highest White blood cell count and mean corpuscular hemoglobin count. Serum result showed that globulin significantly decreased while albumin significantly ($P<0.05$) increased with bitter leaf extracts dosage. Furthermore, 100ml\L broilers had the lowest ($P<0.05$) serum urea and creatinine. The 50ml/L had the highest AST and ALP value and 100ml/L had the highest value. Organ parameters showed that bitter leaf reduced ($P<0.05$) heart weight in a dose-dependent manner. It can be concluded that oral dosage of bitter leaf through water can be used for broilers at the rate of 50ml/L without any negative effect.

Key words: Blood, bitter leaf extracts, feed additive, phytogenics, broiler chickens

Running title: Finisher broiler chickens offered bitter leaf extracts in drinking water

Indices hématologiques, sériques et organiques de poulets de chair en finition recevant des extraits de feuilles amères (Vernonia amygdalina) dans leur eau de boisson

Résumé

Les antibiotiques sont des additifs couramment utilisés pour traiter diverses infections qui menacent la production avicole. Cependant, l'interdiction des antibiotiques dans la plupart des pays développés, en raison de leur résistance antibactérienne chez les poulets et de leurs effets négatifs sur la santé humaine, a nécessité une alternative. Exploiter le potentiel des plantes comme phytogènes est devenu une option. Cette étude a donc été menée pour étudier le rôle des extraits de feuilles amères sur la santé, le développement des organes et les paramètres hématologiques et sériques des poulets de chair. Quatre-vingt-dix poussins de chair Cobb âgés de 500 jours ont été répartis aléatoirement entre trois traitements (0, 50 et 100 ml par litre d'eau de boisson) d'extraits de feuilles amères. Les traitements ont été répétés trois (3) fois, avec 10 oiseaux par réplicat, dans le cadre d'une expérience à conception entièrement randomisée (CRD). L'étude a débuté en phase de finition et a duré 4 semaines. Les résultats hématologiques ont montré que les oiseaux ayant reçu 50 ml/l d'extraits de feuilles amères présentaient les taux de globules blancs et d'hémoglobine corpusculaire les plus élevés. Les résultats sériques ont montré une diminution significative de la globuline, tandis que l'albumine augmentait significativement ($p < 0,05$) avec le dosage des extraits de feuilles amères. De plus, les poulets de chair ayant reçu 100 ml/l présentaient les taux sériques d'urée et de créatinine les

plus faibles (p < 0,05). Le dosage de 50 ml/l présentait les valeurs d'AST et de PAL les plus élevées, tandis que le dosage de 100 ml/l présentait les valeurs les plus élevées. Les paramètres organiques ont montré que les feuilles amères réduisaient (p < 0,05) le poids du cœur de manière dose-dépendante. On peut conclure que l'administration orale de feuilles amères dans l'eau peut être utilisée chez les poulets de chair à raison de 50 ml/l sans aucun effet indésirable.

Mots clés: Sang, extraits de feuilles amères, additif alimentaire, phytogènes, poulets de chair
Titre courant: Poulets de chair en finition recevant des extraits de feuilles amères dans leur eau de boisson

Introduction

Animal protein is very necessary in human diets because of its high biological value in meeting the needed amino acids in human nutrition (Leroy *et al*. 2023). The challenge of high cost of feed and other livestock rearing inputs like medications tend to make meat almost not affordable to large portion of the population (Camillus *et al*., 2023). However, poultry birds still remain a relatively affordable source of animal protein especially broiler chickens (Singh *et al*., 2022). This could be as a result of their suitability to various environments in terms of body size, relative high stocking density per rearing space and management alternatives that made cost tolerable to farmers and consumers. Broiler chickens reach table size within six weeks; at times left till seven weeks or more for those that have preference for relatively tough meat (Animal Welfare, 2019). Commercial rearing of broiler chickens has good returns due to relatively higher turnover rate compared to layers and the nutritional acceptability of its meat. Generally, poultry chickens suffer no taboo or any negative religious disbelief about their consumption. Consumption of broiler chickens is gradually becoming part of daily diets, now that much awareness had been created on their rearing for family consumption and income. The outbreak of Avian Influenza (Bird flu) in 2006 was major devastating experience to the poultry industry in Nigeria (Alhaji *et al*., 2023). The outbreak is still impacting negatively on the industry. This experience therefore prompted the majority of the poultry farmers to deploy various indigenous knowledge on ethnoveterinary practices into prevention and treatment of poultry diseases (Jamil *et al*., 2022). Ethnoveterinary practices involve the use of herbs, generally referred to as phytogenics. The phytogenics are of plant origin incorporated into either feed or water of the birds to enhance productivity, nutrient absorption and elimination of pathogens in the gut of the birds (Irivboje *et al*., 2021). The use of these plant parts had been found effective as antioxidants with therapeutic and prophylactic properties (Edo, 2023). Hence, birds performance were enhanced with ease of preventing common poultry diseases like coccidiosis (Pop *et al*., 2019), Newcastle disease (Irivboje *et al*., 2021) and worm infestation (Acorda *et al*., 2019). Intensive poultry production continually seeks sustainable strategies to enhance performance and overall health in broiler chickens. Bitter leaf has the potential of enhancing performance and health of poultry chickens. It has been reported that bitter leaf (*Vernonia amygdalina*) contain significant quantities of lipids, carbohydrates, proteins having high essential amino acid scores, fibre, iron, phosphorus, copper, calcium potassium, cobalt and manganese, appreciable amounts of biologically active compounds like ascorbic acid, saponins, alkaloids, steroids terpenes, flavonoids, coumarins, ligans, phenolic acids, edotides, xanthones, anthraquinone and sesquiterpenes and caroteinoids (Edo *et al*., 2023). Extracts from bitter leaf can be used as tonics to treat variety of ailments and maladies, including emesis, nausea, diabetes, anorexia, diarrhea, dysentery and other gastrointestinal tract issues (Adebukola *et al*., 2022). Bitter leaf (*Vernonia amygdalina*) extracts has garnered attention for its potential health

benefits in animals due to its rich bioactive compounds (Ajayi *et al*., 2019). Bitter leaf (*Vernonia amygdalina*) is one of the medicinal shrubs common in Nigeria. It is also an edible vegetable used mostly to cook soup. While the bitter leaf plant is useful to man, it has a lot of antioxidants and therapeutic properties that are of importance to animals, especially poultry (Oyeyemi *et al*., 2018). The reports of Oyeyemi *et al*., 2018 and Jamil *et al*., 2022 have highlighted the positive influence of bitter leaf extracts on the growth and health performance of poultry. Phytogenics like bitter leaf extracts as feed additive added to broiler drinking water to improve health, reduce morbidity and enhance production efficiency. These findings suggest that the bioactive components present in bitter leaf may contribute to improved feed utilization and nutrient absorption. However, the specific impact of bitter leaf extracts in the drinking water of broiler chickens remains relatively unexplored. In this investigation it was attempted to contribute to the existing body of knowledge by assessing the contributory impact of bitter leaf extracts in drinking water to the health of finishing broiler chickens through organ development, hematological and serum parameters.

Materials and Methods

Description of Study Area

The study was carried out at the Poultry Unit of the Teaching and Research Farm, Olusegun Agagu University of Science and Technology (OAUSTECH) Okitipupa, Nigeria. The OAUSTECH is located within the rainforest zone, Latitude 5^{o} 28' N and longitude 4^{o} 46' E at an elevation of about 200 m above sea level.

Source of Experimental Materials

Fresh bitter leaves (*Vernonia amygdalina*) were harvested from the Teaching and Research Farm, Olusegun Agagu University of Science and Technology (OAUSTECH), Okitipupa, Ondo State, Nigeria. Ninety broiler chicks were obtained from Zartech Hatchery, Ibadan, Oyo State.

Experimental Birds and Managements

The birds were raised in deep litter system open-sided housing where they received uniform care and management. The experiment lasted for eight weeks. Lighting was provided 24 hours daily, while feed and clean cool drinking water were given *ad libitum* throughout the experimental period. Prior to the arrival of the birds, the pens were subjected to thorough washing and disinfected, followed by scrubbing, cleaning, removal of debris and cobwebs. The outer walls of the pens were properly covered with transparent nylon in order to avoid heat loss. Fresh wood shavings were spread to a depth of 5 cm in order to help in conserving heat, particularly during the brooding stage. Charcoal stoves were used as sources of heat during the brooding stage. Other appliances such as drinkers and feeders were also provided. The birds were vaccinated and fed starter diet (Table 1) for four weeks with plain drinking water.

Preparation of Bitter Leaf extracts

Two hundred grammes (200g) of freshly harvested bitter leaves were rinsed thoroughly without squeezing with clean water to remove dirts. The rinsed leaves were put in a bowl containing two litres of clean water and then squeezed to have the leaves extracts. The extracts was constituted into one litre of water at different volume of the extracts to form the treatments.

Experimental Design

Ninety Cobb 500 day- old broiler chicks were randomly assigned to three (3) treatments (0, 50m and 100ml per litre of drinking water) of constituted bitter leaf extracts for treatments 1, 2 and 3, respectively. The treatments were of 30 birds each. The treatments were replicated three (3) times with ten (10) birds per replicate under a completely randomized design (CRD) experiment. The study commenced at the finisher phase. At the finisher phase, the birds were fed *ad libitum* with finisher diet (Table 1) and exposed

to the experimental drinking water without restriction for 4 weeks.

Haematological and serum parameters

At the end of the fourth week of the finisher phase study, two birds per replicate were randomly selected, making a total of six (6) birds per treatment and starved overnight prior to blood collection. The birds were bled with the use of needle and syringe via the neck veins to collect five (5) ml of blood samples were collected into bottles treated with ethylene diamine tetra acetic acid (EDTA) for haematological analysis. The remainder of each blood sample was allowed to coagulate to produce serum for blood chemistry measurements (Ochei and Kolhatkar, 2008). The samples were immediately transported to the laboratory for analysis. The haematological parameters determined were red blood cell (RBC), white blood cell (WBC), blood platelets, packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) mean corpuscular haemoglobin concentration (MCHC), lymphocytes. The haematological parameters were determined by the use of Neubauer haematocytometer (Lamb, 1991). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were computed using the formulae outlined by Jain (1986). Red blood cell (RBC) and total white blood cells determined by the haemo-cytometer method as outlined by Akintunde *et al*. (2017). Samples for biochemical parameters were first centrifuged and decanted. Sigma kits were thereafter used to determine protein, globulins and albumins. Serum aspartic aminotransferase (AST), alkaline phosphatase (ALP) and alkaline aminotransferase (ALT) were determined using the procedure of Baker and Silverton (1985).

Organ parameters

At the end of the fourth week of the finisher phase study, two birds per replicate were randomly selected, making a total of six (6) birds per treatment offered water but starved of feed overnight for organ evaluation. The birds were slaughtered by severing the jugular vein with a sharp knife. Each slaughtered chicken was thoroughly bled, weighed, scalded, defeathered and eviscerated. The visceral organs evaluated were liver, kidney, lungs, heart, gizzard, spleen and pancreas. All the organs measured were expressed in g/kg live body weight.

Statistical analysis

All experimental data were analysed using One-way Analysis of Variance while means were separated by Duncan Multiple Range Test at 95 % level of significance (SAS Inst. Inc., Cary, NC).

Results

Haematology parameters of broiler chickens

The haematological parameters of broiler chickens offered bitter leaf extracts in drinking water is as shown in Table 2. In all the parameters, only the white blood count, blood platelets and mean corpuscular haemoglobin were significantly ($P>0.05$) influenced by the bitter leaf extracts. Their values decrease with increased ml of bitter leaf extracts, with those on 100ml/litre of drinking water having the least values for white blood count, blood platelets and mean corpuscular haemoglobin. The birds on 100ml/litre of drinking water had white blood count and mean corpuscular haemoglobin values to be significantly ($P<0.05$) different from those on 50ml/litre of drinking water and 0ml/litre of drinking water with those on 0ml/litre of drinking water having the highest values for the two parameters. The blood platelets for the birds on 100ml/litre of drinking water was significantly ($P<0.05$) different from those on 50ml/litre of drinking water and 0ml/litre of drinking water while those on 50ml/litre of drinking water and 0ml/litre of drinking water were not significantly ($P>0.05$) different from one another.

Serum Indices broiler chickens

Table 3 showed the serum indices of broiler

chickens offered bitter leaf extracts in drinking water. All the indices except total protein and glucose were significantly (P<0.05) affected by the bitter leaf extracts in drinking water. The birds under 100ml/litre of drinking water had the highest albumin value and was significantly (P<0.05) different from those under 50ml/litre of drinking water and 0ml/litre of drinking water that were not significantly (P>0.05) different from one another. The globulin values for birds under 100ml/litre of drinking water and 0ml/litre of drinking water were significantly (P<0.05) different with those under 50ml/litre of drinking water not significantly (P>0.05) different from those under 100ml/litre of drinking water and 0ml/litre of drinking water. The aspartate transaminase (AST) value for the birds under 50ml/litre of drinking water was significantly (P<0.05) different from those birds under 100ml/litre of drinking water and 0ml/litre of drinking water that were not significantly (P>0.05) different from one another. The Alanine transaminase (ALT) values were significantly (P<0.05) different among the three treatments with values 12.00, 7.00 and 9.00µ/L for 100, 50 and 0 ml/litre of drinking water respectively. The birds under 100ml/litre of drinking water had the least Alkaline phosphatase (ALP) and significantly (P<0.05) differed from treatments 50ml/litre of drinking water and 0ml/litre of drinking water that were not significantly (P>0.05) different from one another. The values for Chloride, Urea and Creatinine showed similar trends among the treatments. Birds under 100ml/litre of drinking water had the least values for the three serum indices of Chloride, Urea and Creatinine that were significantly (P<0.05) different from values under 50 and 0ml/litre of drinking water that were not significantly (P>0.05) different from one another.

Organ parameters of broiler chicken

Table 4 presents the organ parameters of broiler chicken offered bitter leaf extracts in drinking water. In all the considered organ parameters, only heart was significantly (P>0.05) different. The heart weight decreases with the increased level of bitter leaf extracts in drinking water. Birds offered the highest ml (100ml/litre of drinking water) of bitter leaf extracts significantly (P<0.05) had the least heart weight but with similar weight, which was not significantly (P>0.05) different from birds on 0ml/litre of drinking water.

Discussion

The assessment of clinical and health status of broiler chickens can easily be achieved through haematological and serum values. The white blood count, blood platelets and mean corpuscular haemoglobin were the only haematological parameters significantly influenced by the bitter leaf extracts. This reveals unequal impact of the treatments on the haematological parameters of the broiler chickens. Though all the haematological values were within the normal range for healthy chickens (CCAC, 1993; Mirtuka and Rawnsley, 1997), but the decrease in values of the white blood count, blood platelets and mean corpuscular haemoglobin with increased concentration of the bitter leaf extracts in drinking water tends to indicate that the concentration extracts might not necessary need to be as high as the treatment range before the physiological impact is realized on white blood count, blood platelets and mean corpuscular haemoglobin. In fact, it could be observed that the white blood cell and globulin of the highest dosage followed the same pattern by having the lowest value. This suggest that high concentration of bitter leaf in broiler chicken water might affect the animal immune system. Alternatively, it could also be that the bitter leaf actually controlled pathogens that could have triggered the body defense/immune response due to its antimicrobial activities.

Blood urea nitrogen and creatinine are the final product of protein metabolism, and they are often regarded as indicator of renal functions (Song

et al., 2020). In the study, the low level of urea and creatinine in the highest bitter leaf inclusion, suggest that bitter leaf did not affect renal function or reduced skeletal muscle breakdown. Furthermore, creatinine is an indicator of skeletal muscle breakdown, can increase in the blood serum due to heat stress (Abeni *et al*., 2007). Thus, it also indicate that it can help alleviate broiler heat stress even in tropical environment.

The activities of ALT and AST reflect the integrity of hepatocytes and are often used as indicators of liver injuries/infarction. This is because, when liver damages occur, there is leakage of some of these organ specific enzymes beyond the concentration expected in the blood. Although these enzymes are available in the blood at a level, undue increases could suggest a liver damage especially with ALT enzyme (Adegbeye *et al* 2021). Although 50ml/L had the highest AST and ALP value and 100ml/L had the highest value, they were all within the range for normal broiler chicken, which suggest no negative effect of treatment.

Organ parameters

The influence of the treatments on the organ parameters shows that the treatments virtually had similar impact on the organs. Still, the decreasing weight of heart with increased concentration of bitter leaf extracts in drinking water tends to reflect the potential of the extracts in preventing undue tissue organ growth as reflected in the similar weight value realized for treatments 0ml/litre of drinking water and 50ml/litre of drinking water. This partially support the report of Nilawati *et al*., 2023 that bitter leaf extracts has a good effect on internal organs, namely the heart, liver and spleen and also corroborate the earlier study of Daramola *et al*., (2018) on bitter leaf meal, that high level of bitter leaf meal reduces organ weight of broiler chicken. Therefore, bitter leaf extracts does not impact negatively on the organ anatomy of broiler chickens. Notwithstanding, it can be observed from this result that the heart weight of broilers given bitter leaf decreased with increasing supplementation.

Conclusion

This study showed that the application of bitter leaf extracts in broiler chicken water up to 50ml/L did not compromise the health of broiler chicken. Therefore, as alternative to antibiotics, bitter leaf can be used without compromising broiler health.

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Table 1: Composition of starter diet (%) and the experimental finisher diet (%)

	Starter	**Finisher**
Ingredients	**Composition (%)**	**Composition (%)**
Maize	62.40	66.00
Soybean	27.90	29.70
Bone meal	2.80	2.06
Fish meal	2.48	0
Palm Kernel cake	0	0.45
Groundnut cake	3.60	0
Broiler premix	0.25	0.25
Limestone	0	0.96
Methionine	0.23	0.18
Lysine	0.22	0.13
Common salt	0.16	0.25
Total	**100**	**100**
Metabolizable Energy (ME)	2750kcal/kg	2750kcal/kg
Crude Protein (%)	20	19

Table 2: Haematological parameters of broiler chickens offered bitter leaf extractss in drinking water

Parameters	0ml\L	50ml\L	100ml\L
Pack cell volume (%)	38.00±1.00	36.00±1.00	37.00±1.00
Haemoglobin (g\L)	12.70±0.05	12.00±1.00	12.30±1.05
Red blood count(M\L)	4.00±0.35	3.70±0.05	3.90±0.20
White blood count($x10^9$\L)	19.80±0.15[b]	20.50±0.05[c]	19.40±0.15[a]
Blood platelets (mcl)	32.40±1.05[b]	31.60±0.05[b]	29.10±1.10[a]
Mean corpuscular volume (fi)	95.00±2.00	97.30±1.10	94.90±0.25
Mean corpuscular haemoglobin (pg\dl)	31.80±0.10[b]	32.40±0.05[c]	31.50±0.10[a]
Mean corpuscular haemoglobin concentration (g\dl)	33.40±0.20	33.30±0.10	33.20±0.05

Mean±Standard Deviation

[abc] Means with different superscripts along the rows are significantly different ($p<0.05$)

Table 3: Serum indices of broiler chickens offered bitter leaf extracts in drinking water			
Parameters	0ml\L	50ml\L	100ml\L
Total Protein (g\L)	80.00±5.00	77.00±2.00	78.00±3.00
Albumin (g\L)	34.00±2.00 [a]	34.00±1.00 [a]	38.00±2.00 [b]
Globulin (g\L)	46.00±2.00 [b]	43.00±1.00 [ab]	40.00±2.00 [a]
Aspartate transaminase (AST) (µ\L)	7.00±1.00 [a]	10.00±1.00 [b]	7.00±1.00 [a]
Alanine transaminase (ALT) (µ\L)	9.00±1.00 [b]	7.00±1.00 [a]	12.00±1.00 [c]
Alkaline phosphatase (ALP) (µ\L)	19.80±0.20 [b]	21.30±1.30 [b]	17.60±1.10 [a]
Glucose (mmol\L)	5.90±1.05	7.00±1.00	6.00±0.20
Chloride (mmol\L)	93.00±1.00 [b]	94.00±2.00 [b]	89.00±2.00 [a]
Urea (mmol\L)	3.70±0.10 [b]	3.90±0.15 [b]	2.70±0.05 [a]
Creatinine (mmol\L)	70.10±9.45 [b]	71.30±0.70 [b]	50.10±0.60 [a]

Mean±Standard Deviation

[abc] Means with different superscripts along the rows are significantly different (P<0.05)

Table 4: Organ parameters of broiler chicken offered bitter leaf extractss in drinking water

Parameters	0ml\L	50ml\L	100ml\L
Live Weight (Kg)	2.13±0.13	2.20±1.17	2.28±0.08
Liver (g/Kg live weight)	14.39±1.88	13.83±1.49	14.88±1.61
Kidney (g/Kg live weight)	2.65±0.93	2.35±1.08	1.30±0.95
Gizzard(g/Kg live weight)	18.95±1.74	18.30±2.50	17.79±2.95
Drumstick(g/Kg live weight)	92.61±15.84	89.45±9.89	115.85±12.72
Thigh (g/Kg live weight)	112.40±10.15	120.30±12.87	105.77±13.47
Heart (g/Kg live weight)	5.10^{b}±0.94	4.29^{ab}±0.67	3.54^{a}±0.44
Lung (g/Kg live weight)	4.85±0.22	4.82±1.24	5.54±0.18
Pancreas (g/Kg live weight)	1.26±0.27	1.17±0.50	1.19±0.02
Spleen (g/Kg live weight)	0.66±0.09	0.81±0.12	0.70±0.21

[a,b]Means with different superscript along the rows are significantly (P<0.05) different