Research Article

Effects of *Eichhornia crassipes* Leaf Meal as Feed Additive on Performance Characteristics and Serum Biochemistry of Broiler Birds

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Abstract | This study was carried out to evaluate the performance characteristics, physiological response and carcass quality of broiler birds fed diets containing Water hyacinth (*Eichhornia crassipes*) leaf meal (ECLM) as a feed additive. Treatment diets consisted of a commercial starter and finisher feed with ECLM added at graded levels of 0,1,2,3, and 4g/100kg, T_1 with 0g ECLM served as the control. Two hundred and twenty-five Arbor acre-day-old chicks were randomly assigned to the dietary treatments each replicated thrice for eight weeks. Results revealed that average body weight gain, average daily feed intake, and feed conversion ratio were significantly ($p<0.05$) affected by ECLM while spleen and bursa of fabricius were not significantly ($p>0.05$) affected by ECLM feed additive. Measured serum total protein of the groups on ECLM were significantly ($p<0.05$) higher, while serum cholesterol were significantly ($p<0.05$) lower than those on control group. Birds on 1% and 2% ECLM inclusion had the best dressed weight which was significantly ($p<0.05$) higher than those on control. ECLM inclusion as feed additive increased populations of beneficial bacteria such as *Lactobacillus spp*. and decreased populations of potentially harmful bacteria such as *Escherichia coli* in the gut. No mortality was recorded during the experiment. In the main, ECLM can be a potential feed additive with 1-2% inclusion preferred in broiler production without any negative impact on performance, serum blood parameters, and carcass quality.

Keywords | Broiler, Carcass characteristics, Feed additive, Growth performance, Serum biochemical, Water hyacinth

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INTRODUCTION

Feed constitutes about 65-75% of the total cost of animal production in an intensive system (Aderemi, 2020). Feed additives are substances added to feed to meet a specific purpose. It may enhance the nutritional value, sensory value, or shelf life of feed (Wenk, 2003; Olayemi et al., 2017). They are included in the finished feed product during production, processing, packaging, and storage of feed without being a major ingredient (EFSA, 2018). Broiler production plays a crucial role in meeting the increasing demand for poultry meat. However, the cost of conventional feed ingredients poses a significant challenge for the industry. Exploring alternative feed additives derived from agricultural waste will not only reduce production costs but also contribute to waste management, promote utilization of underutilized resources, and environmental sustainability. Broiler production has witnessed an increased interest in natural and alternative feed additives to enhance production performance and overall bird health (Olaseinde

and Aderemi 2023, Aderemi et al., 2018).

The utilization of plant-based feed additives has gained significant attention in the poultry industry due to their potential to enhance growth performance, improve feed efficiency, and promote overall health in broiler birds. Tham and Udéh (2013) reported that *Eichhornia crassipes* leaf meal (ECLM) influenced the digestibility of nutrients in cows. *Eichhornia crassipes*, commonly known as water hyacinth, is an aquatic plant widely distributed in tropical and subtropical regions. It is known for its rapid growth and high biomass production, making it a promising candidate for utilization in various applications, including animal nutrition (Malik et al, 2016). *Eichhornia crassipes* thrive in environments with high temperatures and low salinity. The plant has a single stalk that holds flowers with colors ranging from pink to purple, thick, broad, and ovate leaves, and can grow as high as 1 meter above the surface of the water. Its leaves can grow up to 10 to 20 cm across its stem (Abdel-sabour, 2010).

Patil (2012) and Carvalho et al. (2017) reported that ECLM is a good source of protein, carbohydrates, vitamins, minerals, and bioactive compounds that can contribute to the nutritional value of broiler diets. Furthermore, ECLM possesses various bioactive components such as flavonoids, phenolics, and antioxidants, which have been reported to exhibit health-promoting effects in animals (Lareo and Bressani 1982, Su et al., 2018). According to Aderemi and Alabi (2013) leaf meal does not only serve as a protein source but also provides some necessary vitamins, minerals, and also oxy carotenoids which are responsible for the yellow color of broiler skin, shank, and egg yolk. Water hyacinth is an invasive aquatic plant that can cause ecological problems in water bodies as reported by Chang et al. (2013). It has not only invaded water bodies, causing anoxia in the water, but it also obstructs rivers and provides a favorable breeding ground for mosquitoes, which seriously endangers farmland, irrigation channels, drainage, water transportation, and human health (Villamagna and Murphy, 2010, Ndimele et al., 2011, Patel, 2012).

Utilizing *Eichhornia crassipes* as a feed additive for broilers present an opportunity for sustainable management of this plant. Harvesting and using water hyacinth as a feed ingredient, would help remove it from water bodies, thereby controlling its spread and minimizing its negative impact on the environment (Wimalarathne & Perera, 2019). The inclusion of ECLM in the diet has been reported to improve body weight gain, feed conversion ratio, and overall performance of broiler birds. However, it is important to note that the optimal inclusion level of ECLM in broiler diets may vary depending on factors such as the bird's age, diet formulation, and environmental conditions. The variability in the inclusion level based on the aforementioned factors and others as necessitate the need for this study. Evaluating the effect of gut microbial population will add to existing information on the utilization of *Eichhornia crassipes* as a feed additive in poultry production. Thus, this study aimed to evaluate the nutritional benefits of ECLM as a feed additive in broiler production.

MATERIALS AND METHODS

Experimental Site

The procedures for this study were carried out at the Animal Science Teaching and Research Farm of Niger Delta University, Amassoma, Bayelsa State. Bayelsa State is located in Southern Nigeria in the core of the Niger Delta Region. The use of animals in this study were in accordance with the national or institutional guidelines for the care and use of laboratory animals and under strict guidelines with the rules and regulations of Ethics Committee of the Institution.

Diet composition

The water hyacinth samples used were gathered from drainage channels within the university campus and were subjected to sun drying over a period of 7 days before being subjected to oven drying at 20°c in the laboratory until a constant weight with 10% moisture content was achieved as described by Malik et al. (2013). The milled form was later incorporated into commercial diets in both starter and finisher phases at graded levels of 0,1,2,3, and 4g/100kg of feed.

Proximate Analysis

Proximate analysis of ECLM was carried out to determine the dry matter (DM) content, crude protein (CP), crude fiber (CF), ether extract (EE), and ash content according to AOAC, (2016). The nitrogen-free extract (NFE) was calculated using values obtained from the proximate composition.

Experimental design

A total of 225 unsexed abor acre plus broilers of day old (50 ± 1.0g) were randomly allocated to five dietary treatment groups (T_1-T_5), the diet without ECLM served as the control (T_1), while inclusion levels 1,2,3, and 4g/100kg were T_2, T_3, T_4 and T_5 respectively. Each group was replicated thrice and the feeding trial lasted for eight weeks. A deep litter-type housing system was made available by the University for this Experiment. The poultry house consisted of 3 rows of individual pens on each side of the complex and 15 pens were allotted for this experiment. The poultry house and its surroundings were thoroughly disinfected with pesticides and germicides a week prior to the arrival of the birds. Feeders and drinkers were thoroughly washed and cleaned daily before being placed in the pens.

The brooding period (acclimatization) lasted a week before the birds were divided into their various feeding groups and the feeding trial commenced. All the necessary routine and occasional management, vaccinations, and other precautions and sanitary measures were also taken throughout the study period as recommended by Oluyemi and Roberts (2000). The birds had access to feed and water *ad-libitum* imploring a completely randomized design.

Blood Analysis

At day 56 of the feeding trial, five (5) birds were randomly selected from each treatment. Blood samples were collected via wing veins. Blood samples for heamatological profile were collected into tubes containing an anticoagulant (ethylene diamine tetra- acetic acid) while blood samples for serum analyses were collected into plain bottles, centrifuged and serum was stored at -20°c until further analysis. Cholesterol, albumin, total protein, creatinine, and globulin were measured from serum as described by Alabi et al. (2017), Olaseinde and Aderemi, (2023).

Microbiological Analysis

On day 56, samples for intestinal microbial loads were collected. Birds (n = 30, 6 birds per treatment) were randomly selected, killed, and ceca contents were collected into sterile container. Contents for each birds was thoroughly mixed and subsequently subjected to microbial counts (Olayemi et al., 2017).

Carcass and internal organ analysis

At the end of the feeding trial, the birds were starved of feed overnight but were allowed access to water. The live weight of birds was taken and five birds per treatment were sacrificed by incising the jugular vein and thoroughly bled until death. The birds were de-feathered, intestines and other internal organs of interest were harvested, weighed, and expressed as a percent of live weight accordingly (Alabi et al, 2017). In addition, the spleen and the bursa of fabricius were also harvested and weighed in respect of the birds' immune systems. The carcass was now cut into various parts head, neck breast, shank, wing, and drumstick.

Statistical analysis

Using SAS [SAS, 2010], all data were submitted to a one-way ANOVA and a least significant differences (LSD) analysis to see if there were any significant differences in the means.

RESULTS AND DISCUSSION

Table 1 showed the proximate composition of the ECLM. It was seen to have high crude protein of 18.08%, fibre of 15.34%, fat of 4.09%, ash 3.54% while the Nitrogen free extract content was 44.28%. The crude protein of ECLM from this study was lower than the findings of Alkassar and Al-shukri (2018) who reported 23.82%CP. This however might be due to processing or analysis method or might be caused by the difference in the potential biotic and abiotic factors present in the water where they grow (Dumaup and Ampode, 2020). The gross composition of the basal diet is shown in Table 2, while Table 3 shows the performance characteristics of the experimental birds. The feed intake of the experimental birds revealed those fed control diet (T_1) had feed intake which was significantly ($p<0.05$) higher than other treatment groups. As the level of inclusion of ECLM increased the feed intake reduced, there is an inverse relationship between the ECLM and the feed intake of the birds. The body weight of those fed on (T_2) 1% ECLM inclusion was significantly higher ($p<0.05$) when compared with others.

Table 1: Proximate composition of (*Eichhornia crassipes*) leaf meal (ECLM)

Dry matter	85.33%
Crude protein	18.08%
Crude fiber	15.34%
Ether extract	4.09%
Ash	3.54%
Nitrogen free extract	44.28

Table 2: Gross composition of basal diet fed to experimental birds

Ingredients	**%**
Maize[a]	35
Palm kernel cake[a]	25
Wheat bran[a]	8
Groundnut cake[a]	25
Fish meal[a]	3
Oyster shell	1.5
Bone meal	2
Vitamin premix[b]	.25
Salt	.25
Calculated CP%	20.54
ME Kcal/kg	2743.2

[a] Calculated values
Metabolizable Energy (ME) = 2743.3kcal kg^{-1}
Crude protein (CP)% = 20.54%
Crude fibre (CF) = 4.98%
Ether Extract (EE) = 5.5%
[b] Premix to provide the followings per kg of feed: Vitamin A - 500 iu, Vit.D3 - 1200 mg, Vit.E - 11 mg, Vit.K3 - 2 mg, Riboflavin - 20 mg, Nicotinic acid - 10 mg, Panthothenic acid - 7 mg, Cobalamin - 0.08 mg, Choline chloride - 900 mg, Folic acid - 1.5 mg, Biotin - 1.5 mg ,Iron – 25 mg, Manganese - 80 mg, Copper - 2 mg, Zinc – 50 mg, Cobalt - 1.2 mg and Selenium - 0.1 mg.

Table 3: Performance Parameters of Experimental Birds (kg)

Parameter	T_1	T_2	T_3	T_4	T_5
IW(g)	50.04±1.11	50.05±0.16	50.15±0.30	51.23±0.05	51.24±0.05
FW(kg)	3.08±0.58^{b}	3.18±0.15^{a}	2.75±0.16^{b}	2.50±0.10^{c}	2.40±0.55^{d}
AWG(kg)	3.03±5.19^{b}	3.13±4.55^{a}	2.70±5.8^{b}	2.45.22±5.8^{c}	2.35±4.32^{d}
ADFI (g)	153.01±41.50^{a}	152.22±30.64^{ab}	147.44±28.22^{c}	138.30±34.22^{d}	131.65±32.43^{d}
FCR	2.78±0.05^{d}	2.68±5.40^{d}	3.0±5.40^{c}	3.09±0.02^{a}	3.07±6.80^{ab}

^{a,b,c} means with the same superscripts along a row are not significantly ($p>0.05$) different from each other.
Where; IW- Initial weight, FW- Final weight, AWG- average weight gain, ADFI-average daily feed intake, FCR- feed conversion ratio.

Table 4: Carcass characteristics of birds fed experimental diets

Parameter (g)	T1	T2	T3	T4	T5
Dressed weight	988.00±0.00^{b}	1120.50±392.50^{a}	1254.50±426.50^{a}	980.50±102.50^{bc}	883.50±255.50^{c}
Head	43.50±0.00	45.50±2.00	45.00±2.50	38.50±5.00	35.00±8.50
Neck	56.00±0.00^{c}	69.00±19.00^{b}	75.00±13.00^{a}	54.50±1.50^{c}	51.50±5.50^{c}
Wings	138.00±0.00^{b}	142.50±56.50^{b}	188.00±10.00^{a}	135.00±1.00^{bc}	133.00±2.00^{c}
Breast	163.00±0.00^{b}	172.50±19.50^{a}	173.00±16.00^{a}	167.00±19.00^{b}	142.00±11.00^{c}
Back	243.00±0.00^{a}	208.50±43.50^{bc}	247.00±35.00^{a}	199.50±57.50^{c}	185.50±4.50^{d}
Shank	75.50±0.00^{ab}	65.00±24.50^{c}	80.50±10.00^{a}	59.00±12.50^{d}	43.50±4.00^{e}
Thigh	175.50±0.00^{b}	184.00±20.50^{a}	186.50±32.00^{a}	152.00±36.50^{c}	118.00±1.50^{d}
Drumstick	136.00±0.00^{c}	159.00±19.900^{b}	173.00±37.00^{a}	134.50±21.50^{c}	111.00±3.00^{d}

^{a,b,c} means with the same superscripts along a row are not significantly ($p>0.05$) different from each other.

Table 5: Serum Biochemical characteristics of experimental birds

Parameter	T1	T2	T3	T4	T5
Total protein g/dl	65.5±13.5^{b}	72.0±16.0^{a}	66.5±1.5^{ab}	66.0±7.0^{ab}	67.5±5.5^{ab}
Albumin g/dl	15.5±2.5	17.5±2.5	17.0±2.0	14.0±2.0	15.5±0.5
Globulin g/dl	54.0±11.0	54.5±13.5	54.0±5.0	50.0±5.0	47.0±5.0
Urea mg/dl	1.45±0.6	1.6±0.3	1.7±0.2	1.95±0.05	1.25±0.45
Creatinine mg/dl	33.5±10.5^{a}	30.0±6.0^{b}	28.0±1.0^{b}	36.5±0.5^{a}	25.0±7.0^{b}
Cholesterol mg/dl	212±13.5^{a}	190.5±17.7^{b}	185.7±16.4^{b}	178.6±18.9^{c}	170.8±14.6^{d}

^{a,b,c} means with the same superscripts along a row are not significantly ($p>0.05$) different from each other.

Table 6: Organ Weight of Experimental Birds

Parameter (g)	T_1	T_2	T_3	T_4	T_5
Lungs	6.00±2.63	8.00±1.12	9.00±5.46	14.00±5.48	10.48±0.62
Liver	28.42±2.11^{d}	30.00±1.2^{b}	32.00±13.1^{a}	34.50±0.70^{b}	36.00±1.40^{a}
Heart	6.46±0.68	9.00±2.73	8.50±2.11	13.49±0.70	15.00±1.01
Gizzard	34.00±6.05^{cd}	34.00±5.62^{cd}	35.00±13.54^{c}	48.00±2.80^{a}	42.00±7.07^{b}
Intestine	160.5±27.58^{b}	154.00±5.52^{c}	142.00±1.40^{d}	156.48±6.30^{c}	170.40±3.50^{a}
Proventriculus	12.00±4.20	13.00±4.20	6.00±2.73	11.00±1.62	14.00±2.63
Spleen	0.06±0.01	0.07±0.02	0.08±0.02	0.07±0.01	0.07±0.02
Bursa of Fabricius	0.17±0.03	0.19±0.02	0.18±0.03	0.19±0.02	0.20±0.01

^{a,b,c} means with the same superscripts along a row are not significantly ($p>0.05$) different from each other.

The feed conversion ratio of T_1 and T_2 are the best, and are significantly different ($p<0.05$) as compared to other groups, with T_5 having the highest value. This study showed that broilers fed diets containing ECLM additive had improved growth performance, perhaps indicating ECLM as a beneficial feed additive at 1-2% level of inclu-

sion. The high protein content of the leaf meal may have contributed to improved growth performance. However, this contradicts other studies which have reported no significant ($p>0.05$) effects or even reduced performance in broilers fed diets containing *Eichhornia crassipes* leaf meal. These discrepancies may be attributed to variations in the composition of the leaf meal, inclusion levels, processing methods, and overall diet formulation. Alkassar and Al-Shukri (2018) reported that excessive amounts of tannin in ECLM resulted in improper digestion of some minerals necessary for metabolism, which eventually led to a decline in the growth rate.

Water hyacinth leaves contain certain anti-nutritional factors that can affect the digestibility and performance of broiler birds. Substances like tannins, phytic acid, and oxalates are present in *Eichhornia crassipes*, which can interfere with nutrient absorption and utilization (Carvalho et al., 2017). High levels of these anti-nutritional factors may lead to reduced performance, hindered nutrient utilization, and negative effects on the digestive system of broilers. Therefore, proper processing techniques such as drying, grinding, and heat treatment may be required to reduce the levels of these anti-nutritional factors and enhance the overall nutritional value of water hyacinth leaf meal.

The results for feed intake show a continuous reduction in consumption with higher levels of inclusion. This could be due to several reasons, including feed texture as adduced by Mingbin et al. (2015), and Xu et al. (2015) who reported that birds prefer crumbs or pellets to finely ground feed. Also, the palatability of the feed was implicated though birds have a relatively low taste bud count of about 70 compared to mammals which have over a thousand. Liu et al. (2018) reported broilers to have more taste buds than egg-type chicken. Table 4 shows the results for carcass characteristics of the experimental birds fed with different levels of ECLM. The dressed weight, the neck, wings, shank, thigh and drumstick of birds fed ECLM at 1 – 2% were significantly ($p<0.05$) higher when compared with those fed control. This result suggests that the addition of ECLM at the studied levels had significant ($p<0.05$) effect on the physiological development of birds up to 2%, perhaps inclusion may have positive effects on meat tenderness and sensory attributes which cannot be ascertained with this study.

Table 5 shows the effect of ECLM on the serum metabolites of broiler, ECLM diets did not significantly affect ($p > 0.05$) the serum total protein, albumin, and globulin levels compared to the control group (T_1). However, the ECLM-treated group exhibited a significant decrease ($p < 0.05$) in serum cholesterol levels, indicating potential hypolipidemic effects. The hypo-cholesterolemic effect observed in this study could be attributed to the presence of bioactive compounds such as phenolics, flavonoids, and phytosterols present in *E. crassipes* leaf meal. These bioactive compounds have been reported to possess antioxidant and lipid-lowering properties. Additionally, the balanced serum biochemistry parameters indicate the safety and tolerability of *E. crassipes* leaf meal as a feed additive in broiler diets. Incorporating ECLM as a feed additive in broiler diets did not adversely affect serum biochemistry parameters. These findings support the potential utilization of *E. crassipes* leaf meal as a natural feed additive in broiler nutrition. Incorporating *Eichhornia crassipes* leaf meal into broiler diets may positively and negatively affect digestibility. The plant's bioactive compounds, such as polyphenols and flavonoids, are believed to positively affect digestibility. The digestibility of nutrients such as crude protein, fat, carbohydrates, and fiber can be influenced by the composition of the leaf meal and the processing methods used. Water hyacinth leaves are known to have a relatively high crude protein content, which can positively affect the digestibility of protein in broiler birds. However, the high fiber content in *Eichhornia crassipes* may negatively affect the digestibility of other nutrients, such as energy.

Table 6 shows that ECLM had a significant ($p<0.05$) effect on some internal organs of the experimental birds, the liver and gizzard of birds fed ECLM were higher than control while the intestine of ECLM-fed birds decreased in size but all were within the normal range for broilers. The result of the organ weight corroborated that of Alabi et al. (2017) and Ayoola et al. (2010) who reported that plant based additives are likely to have hypertrophic influence on organs. This could probably be due to higher physiological activities by these organs triggered by the presence of anti-nutritional factors in ECLM and their concomitant effects. The spleen and bursa of fabricius in this study improved, indicating the possible promotion of the immune system of the broilers. This was in consonance with Olaseinde and Aderemi (2023) who reported large spleen and bursa sizes consequently improved immune systems in broilers fed sprouted pearl millet. Furthermore, ECLM has demonstrated antioxidant and immunomodulatory effects, leading to enhanced immune response and disease resistance in broilers as reported by Dumaup and Ampode (2020).

The inclusion of ECLM in broiler diets has also shown positive effects on serum parameters, indicating improved nutrient utilization, liver function, and overall metabolic health. Table 7 shows the microbial loads observed in the broilers fed the experimental diets. Findings from this study agrees with Kumar et al. (2018) who reported that broilers fed with water hyacinth as a feed additive exhibited increased populations of beneficial bacteria such as *Lactobacillus spp*. and decreased populations of potential-

ly harmful bacteria such as *Escherichia coli*. The findings here could possibly be due to the prebiotic properties of ECLM, including the presence of fermentable fibers, which promote the growth of beneficial bacteria and inhibit the proliferation of pathogens as reported by Olaseinde and Aderemi (2023). The beneficial effects of water hyacinth on microbial loads in broilers can be attributed to several mechanisms. The high fiber content in water hyacinth serves as a substrate for fermentation, promoting the growth of beneficial bacteria. Additionally, water hyacinth contains secondary metabolites such as phenolic compounds and saponins, which possess antimicrobial properties against pathogenic bacteria and fungi.

Table 7: Microbial loads of birds fed experimental diets.

Microbes	T_1	T_2	T_3	T_4	T_5
Escherichia coli	+	+	+	+	+
Proteus Species	+	+	+	+	+
Staphylococcus aureus	+	-	-	-	-
Pseudomonas Species	+	-	-	+	-
Klebsiella Species	+	-	-	+	-
Salmonella paratype. B	-	+	+	+	+
Shigella species	-	-	+	-	-
Lactobacillus specie	-	-	+	+	+

Where + = availability of the microbe in the gut of birds
= non-availability of the microbe in the gut of birds

CONCLUSION

In conclusion, incorporating *Eichhornia crassipes* leaf meal as a feed additive in broiler diets influences the nutrient utilization and overall growth performance. While the high protein content of the leaf meal can potentially improve broiler performance, the presence of anti-nutritional factors may hinder digestibility and nutrient utilization. Therefore, further research is needed to optimize the processing techniques and inclusion levels to maximize the benefits and minimize any negative effects on digestibility and performance characteristics of broiler birds.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

NOVELTY STATEMENT

The study of Microbia loads in the gut of broiler fed Eichhornia crassipes Leaf Meal as Feed Additive is novel and contributed to existing information.

AUTHORS CONTRIBUTION

A. F. A & A. P – Conceptualization of research idea, T. O & E. A –Data collection & Data analysis, A.F.A & A.M.O – Data analysis, Interpretation and writing up.

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