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Studies on the effects of methanolic extracts of wild melon (*Adenopus breviflorus)* on growth response, odour and disease control protocols of finishing broilers

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Abstract

This study evaluated the impact of methanolic extracts from wild melon (*Adenopus breviflorus*) on growth performance, odour and disease control protocols in broilers. The experiment lasted for 42 days, in which 84 broilers were randomly assigned into four treatment groups. Each treatment was further replicated three times, with seven birds per replicate in a completely randomized design (CRD). Control group (T1) received all the necessary routine vaccination throughout their growth stages. Birds on T2, T3, and T4 were given 0.10g of methanolic extract of A. breviflorus in 10mls, 20mls, and 30mls of distilled water respectively. The result revealed that A. breviflorus were able to significantly ($p < 0.05$) improve the body weight gain and feed conversion ratio of the birds on the treatment groups. Odour control parameters showed significant reduction in some odour control parameters, and in disease management, helminthes and Newcastle disease were absent in T1 and T2 while being negligible in T3 and T4 in the experiment. Coccidiosis was absent in all treatments except T4 where it was slightly present. Microbes were present in all treatment groups. In conclusion, the study has revealed that methanolic extracts of A. breviflorus was able to enhance growth performance of the broilers while improving odour control protocols and effectively managing disease incidence.

Keywords: *Adenopus breviflorus*; Broilers; Disease; Extracts; Growth performance; Odour

1. Introduction

Since ancient times, plants and their components have been an essential source of medicine in indigenous poultry production systems. Despite significant advancements in modern medical science, many farmers in Nigeria continue to rely on plant-based remedies and herbal treatments to manage poultry health and odour control (Nworgu et al., 2018). Disease remains the primary challenge in poultry farming in Nigeria, where indigenous poultry production is a critical component of mixed farming systems. The traditional use of medicinal plants is a longstanding practice that is gaining renewed attention. Globally, approximately 20,000 species of higher plants are used for medicinal purposes, with their efficacy attributed to bioactive compounds extracted from raw plant materials, each having unique effects on the body. One of these plant species of interest is *Adenopus breviflorus* which has multi medicinal properties asides its nutritional compositions. *Adenopus breviflorus* is one of such herbs that are traditionally used by livestock farmers for the treatment of diseases in various species of animals (Ajayi *et al.,* 2002). It is a tendril climber. It usually climbs over shrubs and herbs using auxiliary tendrils. It has simple leaves that are veined (Balogun*et al*., 2014). Its fruit is pepo and appear green and has cream-coloured narrow blotches that measures 1-5cm in length with bitter pulp (Kar, 2004). Its seeds always range up to four hundred in an average-size fruit. It has flowers that are radially symmetrical capable of divisions by very longitudinal plane into essentially symmetrical halves and nearly always unisexual (Kazeem, 2014). It belongs to Cucurbitaceae family (Orisakeye*,* 2018). This fruit has traditionally served as a local remedy for managing the health of indigenous chickens. However, there is limited scientific information on the use of *A. breviflorus* as a prophylactic and

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therapeutic agent for poultry diseases and odour control protocols. This study was therefore designed to investigate the effects of A. *breviflorus* of the growth performance, disease and odour control protocols.

2. Materials and Methods

2.1. Location of Study and Duration

This study was carried out in the Poultry unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. The relative humidity ranges from 65-80% while the mean daily temperature ranges from 26.8-31 °C (Agbagha *et al.*, 2000). The rainy season in this part of the country is between the months of April and October and dry season between the months of November and March with annual rainfall range of 1650-1700 mm (Breinholt *et al.,* 1981). The experiment lasted for a period of eight weeks.

2.2. Experimental Animals and Management

The pen was thoroughly washed, cleaned and disinfected before the arrival of the birds. The pens were allowed to dry and wood shavings were spread on the floor of the pens one week before the birds arrived. The experiment involved the use of 84 Abor Acre strain of broilers for a 42 days feeding trial. The birds were purchased from a reputable distributor in Nsukka town. Water and feed were provided *ad libitum*. Heat was also provided according to the environmental temperature during the brooding period. There was strict adherence to medication and vaccination schedule for the control group.

2.3. Preparation of Methanolic Extracts:

Seeds of freshly harvested *Adenopus breviflorus* were collected and air- dried. It was soaked in methanol for 72 hours and thereafter it was filtered by a mesh cloth and the filtrate was concentrated into a greenish-brown syrup mass in a rotary evaporator with a temperature of about 40 ºC under reduced pressure for 3h. The extract syrup formed was left over a water bath for final concentration into solid paste. The concentrate was later reconstituted in sterile distilled water to give the required doses of 0.100 g in 10mls, 20 mls, and 30mls distilled water. The solutions were prepared fresh on the day of experiment prior to the administration. After which different volumes were administered to the birds through their drinking water at different levels.

Table 1 Proximate Composition of the finisher feed (Breedwell) used for the experiment

Feed ingredient	Finisher
Protein	17 % (min.)
Fat	4.00
Fibre	3.50
Calcium	0.80
Av. Phosphorus	0.40
Energy	2900Kcal/Kg(min.)
Lysine	0.88
Methionine	0.37
Salt	0.30
Moisture	14%(max)
Ash	6.5

The ingredients were: maize, SBM, wheat offals, salt, DCP, limestone, methionine, lysine, finisher premix.

2.4. Procedure for Coccidia and Helminths Laboratory Analysis

The faeces were prepared by saturated salt floatation method. About 1 g of the sample was emulsified in 10mls of saturated salt solution. The preparation was filtered using muslin gauze, and then the filtrate was poured into the tube

to the brim and then covered with cover slide. After 30 minutes the cover slide was placed on microscope slide and examined for helminth ova and coccidia cyst.

2.5. Procedure for Newcastle Disease Virus Laboratory Analysis

The serum samples were titrated for Haemaglutination inhibition test using standard Newcastle disease antigen.

2.6. Procedure for Total Microbial Count Laboratory Analysis

The samples were serially diluted ten folds. They were then inoculated into nutrient agar plates and spread out on the surface of the plate. The plates were then incubated at 37 °C for 24 hours. After incubation, the colonies were counted and then the colonies forming unit calculated using the dilutions.

2.7. Protein Determination

2.7.1. Principle

The crude protein content was determined using the micro Kjedahl method. The method is based on the wet combustion of the sample by heating with concentrated sulphuric acid in the presence of metallic and other catalysts to affect the reduction of organic nitrogen in the sample to ammonia, which is retained in solution as ammonium sulphate. The digest having been made alkaline, is distilled to remove ammonia which is trapped and titrated.

2.7.2. Procedure: This involved three major steps:

Digestion: Sample (2 g) was weighed inside Kjeldal digestion flask and 25 ml of concentrated sulphuric acid, and a pinch of digestion catalyst (1 g of copper sulphate, 20 g of sodium sulphate and a pinch of selenium powder) was added. Heat was applied in a fume cupboard slowly at first to prevent undue frothing. Digestion continued for 45 minutes until the digest became clear pale green. This was left until completely cool. Distilled water (20 ml) was added. The digestion flask was rinsed 2-3 times and the rinsing added to the bulk and the volume made up to 50 ml distilled water.

Distillation: Markham distillation apparatus was used for distillation. The distillation apparatus was heated and 10ml of the digest was added into the apparatus via a funnel and allowed to boil. Sodium hydroxide (10ml) of 45% was added from the measuring cylinder. This was distilled into 50 ml of boric acid indicator.

Titration: The alkaline ammonium borate formed was then titrated directly with 0.01NHCl. The litre value which is the volume of acid used was recorded.

%N = Titre X 0.01NHCl X 14.01(At.utN) X 100 X 50 over 1000 X 0.5g X 10 all X 100

%Protein = N X 6.25

2.8. Organic carbon

For carbon 1.0g sample was taken into silica porcelain crucible and dry-ashed in Muffle furnace at 500^0C for 4 hours. Weight of ash was recorded, and organic C content in the sample was calculated by using the formula as given below (Brake, 1992):

Organic C (%) = 100 – (Ash %) over 1.8

Where, factor 1.8 is for converting total organic matter into organic carbon.

Phosphorus and potassium were determined after wet digestion (Ryan et al., 2001). For P determination, colour was developed in the digest and its absorbance was measured using spectronic 21 at 430nm.

Potassium was estimated by placing 1.0ml extract into a test tube and adding 5ml distilled water and 4ml of lithium chloride solution. The tube was shaken and potassium was determined using a flame analyzer (Winkleman et al., 1986).

2.9. Odour Control Parameters

2.9.1. Ammonia

10ml of the sample was pipette into 100ml volumetric flask, 2 ml of 10% potassium tartarate solution was added and it was diluted to 80 ml with distilled water. It was allowed to stand for 25 minutes and the absorption was measured at 410um.

2.9.2. Uric Acid

0.4g of sample was weighed into 150ml round bottom flask, 60ml of ethanol formaldehyde solution was added and refluxed for 1hour, cooled, filtered by sunction through sintered glass crucible and was diluted to 100 ml with ethanol formaldehyde. 20 ml of the sample extract prepared was transferred into 50 ml centrifuge and was centrifuged for 15minutes.the supernatant was poured off and allowed to drain for 10minutes. 20 ml of sodium thiosulphate solution was added to each tube and the precipitate was dissolved by stirring with a glass rod. It was transferred by pipetting 5 ml of this solution into 200 ml graduated flask containing 40 ml succinate buffer solution, and was diluted to 200 ml with water, mixed very well, then the absorbance of the solution was measured at 294nM in 10mm silica cell against blank.

2.9.3. Ammonium Nitrate

1.7 g of the sample was weighed into 500ml and about 100 ml of water was added and then filtered. The volume was made up to 200 ml with distilled water. 15 ml of neutral formalin solution was added, and then 3 drops of phenolphthalein indicator also added. It was titrated with standard sodium hydroxide till solution colour changes from pink to colourless.

2.9.4. Ammonium Sulphate

Reagents used

- Standard sodium hydroxide (0.02m).
- Methyl acid indicator: 0.15g in 500ml water.
- Methyl acid- methylene blue mixed indicator solution.

Equal volume of 0.2% solution of methyl acid was mixed with 0.1% solution of methylene blue in sectified spirit.

2.10. Procedure

10 g of the sample was dissolved and 50ml of cold water was accurately weighed in the volume was filtered and made up to 200 ml

It was titrated with 0.02M sodium hydroxide using 1-2 drops of methyl acid indicator. The colour changed from red to yellow-orange.

It was calculated thus:0.049 X A X M X 100

Where,
A = volume in millilitres of STD NaOH
M= molarity of STD NaOH solution
0.049 = weight of H_2SO_4 that reacts with 1 mole of NaOH (i.e., the M_{2g} weight of H_2SO_4).

2.11. Experimental Design

84 arbor acre plus strain broilers were randomly assigned into four treatment groups of 21 birds per treatment in in a completely Randomized Design (CRD), each treatment were replicated three times with 7 birds per replicate to ensure accuracy in results. The model is as follow;

$$Xij = \mu + Ti + Eij$$

Where;
μ = population mean
Ti = treatment

Eij = Error

2.12. Statistical Analysis

Data collected were subjected to one-way analyses of variance (ANOVA) in a completely randomized design (CRD) using the statistical package for social science (SPSS) version 21. The statistically different means were separated using Duncan's option as found in statistical package/software (Duncan, 1955).

3. Results

Table 2 Growth Performance of Finishing Broilers on Methanolic Extract of *A. breviflorus*

Parameters	T1(Control)	T2(10ml)	T3(20ml)	T4(30ml)	P-value
Initial weight(g)	66.15±0.09	65.980±0.58	66.03±0.02	66.10±0.06	0.98^{NS}
Final weight(kg)	2.36^{c}±0.01	2.69^{a}±0.01	2.51^{b}±0.01	2.65^{a}±0.03	0.00^{S}
Weight gain(kg)	2.30^{c}±0.01	2.62^{a}±0.01	2.45^{b}±0.09	2.59^{ab}±0.01	0.03^{S}
ADWG(g)	54.71^{d}±0.01	62.31^{a}±0.03	58.38^{c}±0.05	61.60^{b}±0.29	0.00^{S}
TFI(g)	5881.43^{b}±0.06	5898.57^{a}±0.58	5838.10^{c}±0.06	5814.29^{d}±0.58	0.00^{S}
ADFI(g)	140.03^{a}±0.02	140.44^{a}±0.29	139.00^{b}±0.06	138.44^{b}±0.17	0.00^{S}
FCR	2.56^{a}±0.06	2.27^{c}±0.03	2.38^{b}±0.01	2.26^{c}±0.02	0.01^{S}
Feed cost/Kg	700^{c}± 1.15	704^{bc} ±1.16	708^{ab}±1.15	712^{a}±1.73	0.01^{S}
FC/Kg Wt Gain	1792^{a}±1.16	1598.08^{d}±1.15	1685.04^{b}±1.73	1609.12^{c}±1.73	0.00^{S}

ADWG = Average Daily Weight Gain, TFI = Total Feed Intake, ADFI = Average Daily Feed Intake, FCR = Feed Conversion Ratio, FC/kg Wt Gain = feed cost per kg weight gain, SEM = Standard Error of Means. Means along the same row with different superscripts are significantly different ($p<0.05$).

T1=control, T2=0.100g of methanolic extract of *Adenopus breviflorus* fruit (MEABF) / 10 mls of distilled water, T3=0.100g MEABF /20 mls distilled water, T4=0.100g MEABF /30 mls distilled water.

In evaluating the weight gain of broiler birds treated with *A. breviflorus*, the T_2 group demonstrated a significantly higher weight gain of 2.62 kg, compared to the control group (T_1), which had the lowest weight gain of 2.30 kg. For feed conversion ratio (FCR), T_2 and T_4 exhibited the most favorable values at 2.27 and 2.26, respectively. These values were similar to each other but significantly better than the FCR of 2.56 observed in the control group. Regarding feed cost per kilogram of weight gain, the control group (T_1) incurred the highest cost at 1792, which was significantly ($p<0.05$) higher than all other treatment groups. T2 recorded the lowest feed cost per kilogram of weight gain at 1598.08, which was also significantly ($p<0.05$) lower than the values for T_3 (1685.04) and T_4 (1609.12).

Table 3 Effect of Methanolic Extract of *Adenopus breviflorus* on Litter Characteristics of the Finishing Broilers

Parameters	T1(Control)	T2(10ml)	T3(20ml)	T4 (30ml)	P-value
Moisture	61.40^{c}±0.23	64.25^{b}±0.14	59.63^{d}±0.06	66.36^{a}±0.58	0.00
PH	8.60±0.17	8.87±0.06	8.63±0.06	8.87±0.06	0.17
Protein (%)	9.34^{b}±0.06	9.71^{a}±0.03	8.38^{c}±0.12	8.26^{c}±0.03	0.00
Ammonia(mg/kg)	3.30±0.06	3.29±0.03	3. 00±0.06	3.58±0.07	0.00
Phosphorus(mg/kg)	8.33^{a}±0.19	8.39^{a}±0.05	6.63^{c}±0.02	7.81^{b}±0.06	0.00
Carbon (%)	2.48^{b}±0.05	2.17^{c}±0.02	2.74^{a}±0.02	2.52^{b}±0.04	0.00
Nitrogen (%)	1.50^{a}±0.03	1.56^{a}±0.02	1.34^{b}±0.02	1.32^{b}±0.01	0.00
Ammonium nitrate(g/kg)	6.50^{a}±0.06	5.69^{b}±0.06	6.96^{a}±0.02	5.99^{b}±0.02	0.00
Ammonium sulphate(g/kg)	27.46^{a}±0.06	24.60^{b}±0.03	28.23^{ab}±0.06	26.36^{ab}±0.03	0.00
Uric acid(g/kg)	35.60^{a}±0.06	30.53^{b}±0.05	30.68^{b}±0.01	30.03^{b}±0.02	0.00

Means along the same row with different superscripts are significantly different ($p<0.05$); T1=control, T2=0.100g of methanolic extract of *Adenopus breviflorus* fruit (MEABF) /10 mls of distilled water, T3=0.100g MEABF /20 mls distilled water, T4=0.100g MEABF /30 mls distilled water.

The analysis of odor characteristics revealed significant differences in several parameters, including nitrogen, ammonium nitrate, ammonium sulfate, and uric acid. For nitrogen percentage, T1 and T2 showed similar values, which were significantly different from the lower values of 1.34% and 1.32% observed in T3 and T4, respectively. In ammonium nitrate, T1 and T3 had significantly higher values of 6.50 g/kg and 6.96 g/kg, respectively, which were themselves similar but significantly ($p<0.05$) different from the other treatment groups. For ammonium sulfate, T1 recorded the highest value at 27.46 g/kg, differing significantly ($p<0.05$) from the values of 24.60 g/kg (T2), 28.23 g/kg (T3), and 26.36 g/kg (T4). For uric acid, T1 exhibited the highest value at 35.60 g/kg, which was significantly ($p<0.05$) different from the values observed in T2 (30.53 g/kg), T3 (30.68 g/kg), and T4 (30.03 g/kg), and which were themselves similar.

Table 4 Disease Control Indices of Finisher Broilers on Methanolic Extract of *Adenopus breviflorus*

Parameters	T1(Control)	T2(10ml)	T3(20ml)	T4(30ml)	SEM	P-Value
Newcastle DV	Nil	Nil	0.12	1.20	0.31	0.05
Helminths	Nil	Nil	Nil	Nil		
Coccidiosis	-ve	-ve	-ve	+ve		
TMC	$1.0x\ 10^{7}$	$1.6x\ 10^{5}$	$2.0x\ 10^{7}$	$3.6x\ 10^{6}$		

T1=control, T2=0.100g of methanolic extract of *Adenopus breviflorus* fruit (MEABF) / 10 mls of distilled water, T3=0.100g MEABF /20 mls distilled water, T4=0.100g MEABF /30 mls distilled water.

In the case of Newcastle disease, no traces were detected in T1 and T2, while T3 and T4 showed minimal traces of Newcastle disease. Helminths were entirely absent across all treatment groups. Coccidiosis was negative in all groups except for T4, where it tested positive while total microbial count (TMC) was observed in all the treatment groups.

4. Discussion

4.1. Growth Parameters

In Tables 2, the effects of methanolic extract of *Adenopus breviflorus* fruits (MEABF) has shown significant differences ($p<0.05$) on growth performance of the broiler chickens. This result corresponds with the earlier work of Irivboje and Olufayo 2024 who reported significant improvement in weight gain and feed conversion ratio (FCR) of broiler chicken when treated with *Lagenaria breviflorus* aqueous extract. However, this result disagreed with the findings of Adeyemo et al., 2022 who reported that there were no significant different when spotted pumpkin (*Lagenaria breviflorus)* extract was administered to broiler chickens. The variations observed in both findings could be as a result of difference in the method of extraction as well as the inclusion levels. The administration of A. *breviflorus* extract in this study significantly ($p<0.05$) affected the average weight gain, final weight gain, and feed conversion ratio with T_2 (0.5%) recording the highest value above the control and other treatment means. This result indicated that at 10ml of *A. breviflorus* extract could serve as a potent phytogenic growth promoter in broiler chicken. This work agrees with the report of Onibi et al. (2009), which asserts that medicinal plants may be used as an alternative to antibiotic growth promoters in poultry feeding because of their antimicrobial properties.

4.2. Odour control effect

Tables 3, represents the MEABF on odour control parameters of broiler birds administered methanolic extract of A. *breviflorus* fruits (MEABF). The effect of MEABF showed significant difference on some odour control parameters such as ammonium nitrate, ammonium sulphate nitrogen, phosphorus and ammonia. These results simply mean that digestibility was improved by the extracts and when digestibility is optimised the nutrients in their feed will be absorbed and utilised by their body hence affecting their growth positively and reducing the quantity of these nutrients in their litter and thereby controlling odour emissions from their houses. This report is in accordance with the findings of Evelien*, (*2014), who reported that when digestibility is optimised, the quality of the litter including moisture and pH will be better. However, the results from this current study total agreement with the findings of Zentner*,* (2011) who reported that saponin, an active ingredient of *Adenopus breviflorus* is phytogenic and has shown potentials to reduce ammonia emissions of animals by inhibiting urease activity that converts urea into ammonia and carbon dioxide. Gut health can improve bird's performances by fully utilizing the nutrients in feed, thereby reducing the amount of undigested nutrients emitted into the poultry house. The presence of undigested proteins and amino acids in the gut favours production of undesired metabolites, such as biogenic amines and ammonia that are then excreted by birds into the environment (Basharat et al*.,* 2015).

4.3. Efficacy to Disease Resistance

Tables 4, represents the effects of Methanolic Extract of *Adenopus breviflorus* fruits. This result has shown that MEABF was effective against Newcastle, helminths, and coccidiosis. These finding collaborates with the work of Nworgu et al., 2018, that *Adenopus breviflorus* has the ability to cure Newcastle diseases, coccidiosis and Helminths in farm anmals. Helminth was not even observed in all the treatments even at varying concentrations of MEABF. These results are in accordance with the findings of Mendoza et al. (2019) who worked with plant additive of *Acacia concinna* that contains saponins in broiler birds and discovered that it can be effective when it is used to prevent coccidiosis in them. Muthamilselvan (2016), observed that herbal products are emerging as a strategy to combat coccidiosis, and most of these products used have been confirmed to contain several phytochemicals like phenolic acids, steroids, alkaloids, terpenoids, tannins and flavonoids (Abbas et al., 2013). Studies by Olayinka et al., (2007) using this same fruit showed it has the ability to inhibit the growth of Gram-positive and Gram- negative bacteria, and that when compared with antibiotic, that it has moderate activity. Ethanolic extracts of this fruit elicited excellent control of *Eimeria* oocyst and *Ascaris galli* (Osuntokun et *al*., 2019) All these phytochemicals are present in *Adenopus breviflorus*. So this fruit may be used very well to combat these diseases that scare farmers away from poultry business.

5. Conclusion

The findings from this research have shown that methanolic extracts of *Adenopus breviflorus* significantly enhanced the growth performance of broilers. The A. *breviflorus* extract significantly ($p<0.05$) reduced several odour control parameters, including ammonia, ammonium nitrate, ammonium sulfate, and uric acid, thereby minimizing odor emissions in poultry pens. More so, in the disease control protocol, no helminths and only minimal levels of Newcastle disease and coccidiosis were observed in treatments T_3 (20 ml) and T_4 (30 ml), compared to the control group (T_1).

Recommendation

I recommend T_2 to farmers for improved profit margins due to its superior growth performance, reduced feed cost per kilogram of weight gain, and advanced protocols for odour management and disease control.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

This study was conducted in accordance with the guidelines for the care and use of animals in research, as approved by the Institutional Animal Care and Use Committee of the University of Nigeria, Nsukka (Approval number: UNN/IACUC/2023/001).

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