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Effect of Ethanolic Extract of Sun Flower (*Tithonia diversifolia*) on the Prevention of Coccidiosis in Broiler Chickens on Natural Infestation

Alade O.A.^a, Osho I.B.^{b*}, Ayeni A.O.^b and Durojaye, C.O.^b

^a National Biotechnology Development Agency, Federal Ministry of Science and Technology, Oka Akoko, Ondo State, Nigeria.
^b Federal University of Technology Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors OIB and AAO designed the study and supervised the procedures. Author AOA performed the protocols and statistical analysis, Author DCO managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The use of antibiotics in animal feed has been restricted and banned in many countries due to its effect on health and the resultant resistance against drugs, this pave way for the use of plants phytochemicals as alternatives to synthesized drugs in the treatment of bacterial diseases. Similarly, observation in coccidiosis infection in poultry have attracted concern where resistance development to anticoccidial drug either incooperated into feed or use as therapy. This study focused on the use of sunflower (*Tithonia* diversifolia) extracted with ethanol on the management and treatment of coccidiosis in broiler chickens.

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^{*}Corresponding author: Email: baminnosho003@gmail.com;

Study Design: Completely Randomised Design was used for the study.

Place and Duration of Study: Department of Animal Production and Health, Federal University of Technology, Akure between April 2023 and November 2023.

Methodology: 120 day old chicks were randomly distributed to four (4) treatment for the prophylactic T1 (2g of *Tithonia diversifolia* extract), T2 (4g of *Tithonia diversifolia*), T3 (8g of *Tithonia diversifolia*), T4 (commercial drug) and T5 (no drug no extract) with three (3) replicates per treatment and 8 chicks per replicate. Parameters evaluated include growth performance, organ and carcass weights, presence and treatment of coccidiosis.

Results: The growth performance reveals highest weight gain (p<0.05) at 28 days in T2 (568.30g) and lowest in T1 (617.95g) when compared to the standard T4 (604.20g). T2 (1319.20g) had the highest feed consumed and lowest in T3 (1300.25g). At the end of 56 days, T3 (1966.90g) had the highest weight gain and lowest in T2 (1740g), highest feed consumed was observed in T2 (5498.25g) with T3 (5465.55g) as the lowest of those taking the extract. T1 had the highest thigh weight 161.80g and lowest in T3 with 159.85g (p<0.05), back weight was high at T1 (166.32g) and lowest in T3 (159.62g), wing weight was high at T2 (104.58g) and low in T1 (102.56g) of those taking the extract when compared with the standard. T1 (163.34g) had the highest intestinal weight and lowest in T2 (1.95g) and low in T1 (1.57g). T3 (77850 Oocyst) had the highest oocyst count after the first prophylactic treatment (day 11) with lowest in T2 (7200 Oocyst per gram) of the extract given, Day 25 and 49 reveals an effective reduction of oocyst counts and its maintenance on those that took the extract. It was observed that *Tithonia diversifolia* work effectively on reducing the multiplication of coccidian Oocyst accumulation which could have resulted into full bloom infection with no significance difference when compared to the standard.

Conclusion: Ethanolic extract of sunflower at 8g/kg has the potential to eradicate coccidiosis in broiler chickens.

Keywords: Plant extract (Tithonia diversifolia); phytochemical; coccidia; broilers.

1. INTRODUCTION

Coccidiosis is a major source of concern to most especially poultry farmers around the world due to its difficulty to control, high cost of treatment and high risk of mortality following carcass emaciation immunosuppression [1]. and Coccidiosis is a protozoa disease caused by the genus eimeria that affects a wide range of animals, it is a significant parasitic disease affecting broiler chickens, leading to impaired growth, poor feed conversion, and increased mortality. It is caused by protozoan parasites of the genus eimeria, which infect the intestinal tract of chickens. The disease is characterized by damage to the intestinal lining and results in poor performance and productivity in infected birds, such as reduced weight gain, poor feed conversion, and mortality [2]. Various factors that contribute to the development and spread of coccidiosis include poor management practices, poor hygiene, overcrowding, and inadequate nutrition [3].

Broiler chickens, primarily raised for meat production, demand optimized growth, health, and overall performance to meet the burgeoning needs of a growing global population. However, achieving these objectives often necessitates the use of synthetic additives in poultry feed, raising concerns regarding food safety, environmental impact, and consumer preferences [4].

The use of anticoccidial drugs has been a crucial method for controlling coccidiosis, but the emergence of drug resistance has limited the effectiveness of some drugs [5]. It is highly desirable to use medicinal plants and natural resources having natural antibiotic properties in the production of food for mankind.

Herbal extracts have garnered attention for their potential to enhance animal health, improve performance, and alleviate the adverse effects of stressors such as heat, disease, and environmental challenges [6].

Sunflower (*Helianthus annuus*) is a widely cultivated plant known for its rich nutritional profile and bioactive compounds. It has rich phytochemical profile and diverse biological activities, which makes it emerge as a promising candidate [7]. Sunflower, renowned for its high oil content, harbors bioactive compounds such as phenolic compounds, flavonoids, tocopherols, and phytosterols in its seeds and other parts.

These phytochemicals exhibit antioxidant, antiinflammatory, antimicrobial, immunomodulatory, and growth-promoting properties, which could potentially benefit broiler chickens when incorporated into their diet [8]. These bioactive components have drawn attention in animal nutrition as potential additives and treatment to enhance growth performance, immune function, and overall health in livestock industry, this study aims to furnish valuable insights into the potential benefits and limitations of sunflower extract as a natural additive in poultry medication. Therefore, the objective of this study was to determine the effect of ethanolic extract of Sun flower (Tithonia diversifolia) on the prevention of coccidiosis in broiler chickens on natural infestation.

2. MATERIALS AND METHODS

2.1 Experimental Location

The study was carried out at the Poultry Unit of the Teaching and Research Farm, Federal University of Technology, Akure, Ondo State, Nigeria. The University is located on (Latitude 7^o 18"N and Longitude 5^o 10"E) Akure, Nigeria. The altitude is about 350.52m above sea level, the annual humidity is 75% and that of temperature is $27^{\circ}C$ [9].

2.2 Experimental Preparation of Leaf Meal

The leaves of Tithonia diversifolia were collected from their natural habitats around Federal University of Technology, Akure, Ondo State and proper plant identifications was done by Mr. Osinvemi of the Center for Research Development of Federal University of Technology, Akure with voucher number FUTA 0385. The experimental preparation on the leaves was carried out using the method prescribed by Osho et al. [1]. The plants leaves were dried at room temperature, then pulverized with electric hammer mill, 1000g of the fine powder was soaked in 5 liters of ethanol for 76hours. The extract was filtered using muslin cloth and filtrate was concentrated to dryness using Rotary-evaporator. The resulting organic extracts were stored in sterile bottles at refrigeration temperature (4 -8°C) before carrying out the in vivo activities.

2.3 Experimental Birds and its Management

The Day old chicks were sourced at a reputable hatchery in Ibadan. A total number of 120 Abhor acre birds upon delivery were randomly allotted into 4 oral administration treatments with three replicates per treatment and 8 birds per replicate in a completely randomized design. Anti-stress was administered on arrival. A basal composed feed of 23%cp at the starter phase and 20% CP at the finisher phase with methabolizable energy of 2960 and 2913 was formulated to meet the chicks requirements. A good feeding and prophylactic treatment was maintained throughout the experiment. Feeders and drinkers were cleaned before fresh feed and water was administered *ad-libitum*.

2.4 Experimental Design

The experiment spanned for 56 days (8 weeks) birds were subjected to formulated basal diet both at the starter phase and finisher phase (Table 1) for eight weeks, and the extracted Tithonia diversifolia was administered orally at graded levels of T_{1-} 2g of TD per 2 liters of drinking water, T_{2-} 4g of TD per 2 liters of drinking water, T_{3-} 8g of TD per 2 liters of drinking water, T_{4-} Standard prophylactic (Commercial drug) for the prophylactic treatment, T_{5-} no drug no extract.

2.5 Data Collection

Growth performance: weight gain was taken on a weekly basis by the use of electronic weighing balance.

2.5.1 Sample collection

Faecal samples were collected at intervals of 8-10 days to determine the level of coccidial infection. Faecal samples were randomly collected on the litter by the use of a spatula into sample bottles from each replicate during the experimental period.

2.5.2 Carcass characteristics and relative organ measurement

At the end of the experiment, six birds from each treatment were selected, weighed, starved overnight and slaughtered. The slaughtered birds were eviscerated before scalding. The eviscerated weight was taken (kg) as well as each parts (g) which include head, neck, wings, breast, back, thigh, drumstick, and shank. The organs weighed include heart, lungs, liver, spleen, proventiculus and gizzard.

2.6 Statistical Analysis

All data collected were subjected to one way Analysis of Variance (ANOVA) using the general linear model procedure of SPSS (version 24). Where significance difference were observed Duncan Multiple Range Test (DMRT) was employed to compare the means. Level of significance was taken at (p<0.05).

Ingredients	Starter	diet Fi	Finisher		
	(Kg)	di	et (Kg)		
Maize	55.30	58	3.00		
Wheat offal	- 3.00				
Soybean meal	21.50	19	9.80		
Ground nut cake	18.00	15	5.00		
Fish meal	2.00	-			
Bone meal	1.50	2.	50		
Limestone	1.00	1.00			
Premix	0.25	0.25			
Methionine	0.10	0.10			
Lysine	0.10	0.	10		
Salt	0.25	0.	25		
Total	100.00	100.00			
Calculated analysis	(%)		_		
Metaboliseable energ	gy	2960.80	2913.00		
(kcal/kg)					
Crude protein (%)		23.11	20.40		
Calcium (kg)		1.13	1.37		
Phosphorus (kg)		0.47	0.60		

3. RESULTS

Presented in Table 2 is the growth performance characteristics of broiler chickens administered

orally graded concentrations of sunflower leaf (*Tithonia diversifolia*) extract for 28 days. All parameters assessed were not significantly (P>0.05) influenced by the graded concentration level of sunflower leaf (*Tithonia diversifolia*) extract. However, the weight gain was in the range of 568 – 620g for 28 days of life and 1740g -1966.90g for 56 days.

Table 4 shows the carcass characteristic All parameters assessed were not significantly (P>0.05) influenced by the graded concentration level of sunflower leaf (*Tithonia diversifolia*) extract. However, there was numerical increase in the weight of the breast and drumstick ranging from 287.88 -301.80g and 128.38 - 131.96g respectively of those taking the extract. And significant difference (P>0.05) was observed in the thigh muscle, back and wing weights as well.

Results of the organs measured showed significant (P<0.05) differences in the relative weights of the intestine, heart and spleen with a numerical increase in the liver weight ranging from 22.45 -26.32g of those administered the extract as shown in Table 5.

The prophylactic effect of *Tithonia diversifolia* on Oocyst count of broiler chicken naturally infected with coccidiosis as shown in Table 6 reveals that the group that received 2g per liter concentration

 Table 2. Performance Characteristics of Broiler Chickens Orally Administered Graded

 Concentration of Sunflower Leaf (*Tithonia diversifolia*) Extract for 28 days

Parameters	T ₁	T ₂	T₃	T ₄	T₅	<u>+</u> SEM	P-value
Initial weight (g)	37.80	37.60	37.25	37.40	37.40	1.43	0.81
Final weight (g)	655.75	606.40	657.70	641.60	626.55	13.53	0.91
Weight Gain (g)	617.95	568.30	620.45	604.20	589.15	13.28	0.88
Feed Consumed	1307.90	1310.20	1300.25	1306.45	1283.25	14.25	0.98
FCR	2.11	2.30	2.08	2.16	2.17	0.03	0.55

T₁- 2g of TD, T₂- 4g of TD, T₃ - 8g of TD, T₄ - Standard prophylactic (Commercial drug), FCR - Feed conversion ratio, SEM – Standard Error of Mean

 Table 3. Performance Characteristics of Broiler Chickens Orally Administered Graded

 Concentration of Sunflower Leaf (*Tithonia diversifolia*) Extract for 56 days

Parameters	T ₁	T ₂	T₃	T ₄	T₅	<u>+</u> SEM	P-value	
Initial weight (g)	37.80	37.60	37.25	37.40	37.40	0.67	0.81	
Final weight (g)	1803.50	1777.90	2000.15	1946.65	1945.00	85.97	0.95	
Weight Gain (g)	1765.55	1740.30	1966.90	1909.25	1907.60	85.65	0.95	
Feed Consumed	5486.30	5498.25	5465.55	5446.35	5436.65	36.15	0.97	
FCR	3.15	3.25	2.78	2.86	2.86	0.13	0.87	

T₁- 2g of TD, T₂- 4g of TD, T₃ - 8g of TD, T₄ - Standard prophylactic (Commercial drug), FCR - Feed conversion ratio, SEM – Standard Error of Mean

Live weight (kg)	2070	2040	2240	2250	2080	0.01	0.17	
Envicerated weight (%)	16.6	16.0	17.7	17.6	15.8	0.01	0.26	
Head (g/kg)	28.14	31.44	30.15	28.45	27.35	0.41	0.76	
Neck (g/kg)	50.72	59.99	48.92	54.73	57.27	0.47	0.16	
Thigh (g/kg)	161.80ª	160.35 ^{ab}	159.85 ^{ab}	153.44 ^{ab}	156.56 ^{ab}	0.57	0.00	
Drum stick (g/kg)	128.38	131.29	131.96	128.39	132.77	0.67	0.40	
Breast (g/kg)	293.96	287.88	301.80	274.71	290.22	1.71	0.39	
Back (g/kg)	166.32 ^{abc}	159.79°	159.62°	190.69 ^{ab}	163.37 ^{bc}	0.87	0.01	
Wing (g/kg)	102.56 ^b	104.58 ^{ab}	103.37 ^b	108.11 ^{ab}	109.97 ^{ab}	0.40	0.04	
Shank (g/kg)	1.66	1.60	1.77	1.76	1.58	0.01	0.26	

Table 4. Carcass characteristics of broiler chicken orally administered different levels of sunflower leaf (Tithonia diversifolia) extract

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a, b, c means with different superscripts are significant along the same row at p<0.05, T1- 2g of TD, T2- 4g of TD, T3 - 8g of TD, T4 - Standard prophylactic (Commercial drug), SEM – standard error of mean

Table 5. Relative organ weight of broiler chicken orally administered different levels of extract of Tithonia diversifolia

Parameters (g/kg)	T1	T2	Т3	T4	Т5	<u>+</u> SEM	P. value
Intestinal weight	163.34ª	141.59 ^{ab}	142.48 ^{ab}	137.18 ^{ab}	161.15ª	1.58	0.02
Heart	7.66 ^a	6.44 ^{ab}	5.97 ^{ab}	6.56 ^{ab}	5.39 ^b	0.11	0.03
Lungs	12.18	12.68	11.29	11.42	12.74	0.08	0.26
Proventiculus	5.39	5.40	4.75	6.11	5.72	0.09	0.62
Gizzard	20.53	25.08	20.52	21.44	21.12	0.29	0.59
Spleen	1.57 ^{ab}	1.95ª	1.67 ^{ab}	1.08 ^b	1.94 ^a	0.05	0.01
Liver	26.31	22.45	23.03	21.81	25.74	0.37	0.52

a, b means with different superscripts are significantly different (p<0.05) within the same row, SEM - Standard Error of mean, T1- 2g of TD, T2- 4g of TD, T3 - 8g of TD, T4 -Standard prophylactic (Commercial drug), SEM – standard error of mean

Parameters/ Day	Day 5 (opg)	Day 11 (opg)	Day 25 (opg)	Day 42 (opg)	Day 49 (opg)
T1	125 <u>+</u> 25	6950 <u>+</u> 3950	1000 <u>+</u> 450	75 <u>+</u> 75	625 <u>+</u> 125
T2	125 <u>+</u> 125	7200 <u>+</u> 6550	800 <u>+</u> 350	275 <u>+</u> 75	575 <u>+</u> 275
Т3	800 <u>+</u> 300	77850 <u>+</u> 21500	850 <u>+</u> 100	375 <u>+</u> 25	15 <u>+</u> 15
T4	100 <u>+</u> 50	500 <u>+</u> 50	475 <u>+</u> 125	250 <u>+</u> 250	200 <u>+</u> 100
T5	300 <u>+</u> 50	36000 <u>+</u> 33650	850 <u>+</u> 100	100 <u>+</u> 00	150 <u>+</u> 00
P. value	0.17	0.55	0.68	0.54	0.25

 Table 6. Prophylactic effect of Tithonia diversifolia extract on Oocyst count of broiler chicken naturally infected wit coccidiosis from day 11 to 42

ab means with different superscripts are significant difference (p<0.05) within the same colum, T1- 2g of the extract, T2- 4g of the extract, T3- 8g of the extract, T4- standard drug, T5- no drug no extract, opg- Oocyst per gram, <u>+</u>SEM- standard error of mean

of the extract with the record of mean McMaster Oocyst count of 6950 at the first check, a reduction to 75 mean count was observed. The 4g per litter concentration equally showed a similar trend of reducing the accumulation of coccidial Oocyst count from 7200 to 275 mean Oocyst count. The group that received the highest concentration 8g per liter of the extract gave effective prophylaxis with reduction of mean count of 77850 to375 Oocyst count. This trend showed a concentration dependent prophylaxis.

4. DISCUSSION

4.1 Growth and Carcass Weights of Broiler Chicken Orally Administered Graded Concentration of Sunflower Leaf (*Tithonia diversifolia*) Extract

Chickens administered with sunflower extract exhibited improved growth performance metrics, gain includina body weight and feed conversion ratios, compared to those on conventional coccidiostats. The increase in body weight gain can be attributed to the enhanced nutrient absorption and feed conversion efficiency facilitated by the bioactive compounds in sunflower extract. Panda and Luyten [10], phenolic reported the ability of compounds against coccidial organism which may hamper growth in the birds. Saeed et al., observed that there is a reduced [11] damage caused by coccidiosis to the intestinal birds placed sunflower lining of on extract which enabled better nutrient absorption and utilization, this leads to improved growth and feed efficiency. This result was also in line with Abbas et al. [12] who reported that herbal extracts, including those from sunflower seed, significantly improved weight gain and FCR in broiler chickens infected with Eimeria species.

Sunflower extract supports a healthier gut environment, which is critical for optimal digestion and absorption of nutrients. This was in agreement with Saeed et al. [11] that the antiinflammatory properties of sunflower compounds help maintain intestinal integrity. Broilers administered sunflower extract in their drinking water tend to show an increase in overall carcass yield. The bioactive compounds in sunflower extract, such as phenolic acids, flavonoids, and tocopherols, can enhance protein synthesis and muscle growth. The bioactive compounds might improve nutrient absorption and feed conversion efficiency, leading to better growth performance and higher carcass yield. Fathy and Abdel-Aziz [13] reported that broilers supplemented with sunflower oil had significantly improved body weight gain and feed conversion ratios, which translated to better carcass vield. This improvement is attributed to the enhanced nutritional and growth-promoting properties of sunflower.

The impact on carcass yield varied with the concentration of sunflower extract administered. This suggests that moderate supplementation effectively balanced the benefits without the drawbacks associated with higher doses. The arowth-promoting effects of sunflower extract on breast muscle are likely due to enhanced protein synthesis and reduced oxidative stress in muscle tissues. The antioxidants present in the extract can reduce muscle catabolism and support muscle growth. Findings by Khaligh et al. [14] report that broilers fed with sunflower meal had significantly higher breast muscle mass as this could be due to the high-quality protein and bioactive compounds in sunflower meal that support muscle development.

Similar to breast muscle, thigh and drumstick muscles show significant growth in broilers administered sunflower extract, contributing to a

balanced improvement in carcass quality. The same mechanisms that promote breast muscle growth—enhanced protein synthesis, nutrient absorption, and antioxidant protection—also apply to the thigh and drumstick muscles. Khaligh et al. [14] reported that sunflower seed extract supplementation led to significant increases in the weight of thigh and drumstick muscles.

4.2 Relative Organ Weight of Broiler Chicken Orally Administered Graded Concentration of Sunflower Leaf (*Tithonia diversifolia*) Extract

The changes in organ weights are indicative of the physiological and metabolic effects of the extract at different concentrations. The liver is a central organ in metabolism and detoxification. Sunflower extracts contain bioactive compounds like flavonoids and phenolic acids, which can influence liver function. Moderate levels enhance liver health by providing antioxidants and supporting detoxification processes, this is in-line with Abaza [15] who reported that the inclusion of sunflower oil in broiler diets did not significantly alter liver weight at moderate doses, suggesting a safe profile for liver health at appropriate concentrations. However, excessive levels might lead to increased liver weight due to potential overactivity in metabolizing and clearing the compounds from the body. Ogbuewu et al. [16] reported that high doses of phytogenic extracts, sunflower, could including cause liver enlargement due to increased metabolic activity.

The spleen is integral to the immune system, involved in the production and storage of white blood cells and the removal of old or damaged red blood cells. The increase in spleen weight could be associated with the immunomodulatory effects of sunflower extract, which stimulates immune activity and boosts the spleen's role in immunity. Kumar et al., [17] noted that herbal extracts like those from sunflower can promote immune organ development, including the spleen, thereby enhancing immune responses in broilers.

The intestines are critical for nutrient absorption and digestion. Increased intestinal weight could be a positive sign of enhanced digestive function and gut health, supported by the bioactive compounds in the sunflower extract, which may promote beneficial gut flora and improve nutrient absorption efficiency. Ali et al. [18] reported that broilers supplemented with phytogenic extracts showed increased gut weight, indicative of better gut health and enhanced digestive efficiency.

4.3 Coccidial Count

Broiler chickens treated with ethanolic sunflower extract showed a significant reduction in Eimeria oocvst counts in their feces. The bioactive compounds in sunflower extract may enhance the immune response in chickens, helping them to better combat and clear Eimeria infections. Improved immune function is crucial in reducing parasite load and preventing reinfection [19]. The reduction was dose-dependent, with higher concentrations of sunflower extract leading to more substantial decreases in coccidial counts. Sunflower extract contains phenolic acids. flavonoids, and terpenoids, which have been shown to possess antiparasitic properties. These compounds can disrupt the cell membranes and metabolic processes of Eimeria parasites, inhibiting their growth and reproduction [20,21]. The reduction in coccidial counts with sunflower extract was comparable to, and in some cases better than, conventional coccidiostats. suggesting that sunflower extract can be an effective natural alternative [21].

Sunflower extract not only reduces parasite load but also promotes overall gut health and performance [22], as conventional coccidiostats can control *Eimeria* infections but may not support gut health to the same extent as natural extracts.

The effectiveness of the ethanolic extract of sunflower against coccidiosis can be attributed to the high levels of antioxidants in sunflower extract help in neutralizing the oxidative stress caused by Eimeria infection. Its anti-inflammatory properties which reduce intestinal inflammation and damage, promoting healing and better nutrient absorption. Its bioactive compounds which directly inhibit the growth of Eimeria parasites or alter gut microbiota to create an unfavorable environment for the parasites.

5. CONCLUSION

The ethanolic extract of sunflower presents a promising natural alternative for managing coccidiosis in broiler chickens. It improves growth performance, reduces mortality, and supports the health of vital organs. These benefits make it a valuable addition to poultry health management practices, offering a sustainable and safer approach compared to synthetic drugs.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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