



Effects of *Moringa Oleifera* Seed Supplemented Diets on *Eimeria tenella* Infected Broiler Birds

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Abstract

Coccidiosis is an important disease affecting poultry industry worldwide. Infection is caused by different *Eimeria* species leading to mortality and economic losses. Increasing drug-resistance, toxicity of most anticoccidial, tight government regulations on the use of anticoccidial drugs coupled with non-acceptability of anticoccidial medication in most commercial poultry production has prompted the quest for alternate therapy. This study was carried out to determine the aqueous seed extracts (AE) and ethanolic seed extracts (EE) of *Moringa oleifera* on *Eimeria tenella* infected chicks. Seventy (70) day-old broiler chicks were purchased from a local poultry dealer in Kaduna, Kaduna State, Nigeria. The chicks were randomly divided into seven groups of ten birds each after seventeen days of brooding. Birds in Group 1 formed the uninfected control group while birds in Groups 2 to 7 were separately infected orally with 2×10^4 sporulated oocyst of *E. tenella* on day 18 and their feeds treated as follows: Group 2: Vital feed with no supplement; Group 3: Vital feed + 0.6g/kg Amprolium; Group 4: Vital feed + 1g/kg AE, Group 5: Vital feed + 2g/kg AE; Group 6: Vital feed + 1g/kg EE, and Group 7: Vital feed + 2g/kg EE. Anticoccidial effects of *moringa* seed was conducted on the basis of oocyst output per gram of faeces, mortality rate, caecal lesion score, feed intake and live weight gain. Phytochemical screening of *M. oleifera* seeds showed the presence of alkaloid, glycoside, flavones, saponins, tannins, terpenoids and phenolic compounds. Although both AE and EE exhibited some level of anticoccidial efficacy when compared with infected and untreated control birds, the efficacy of amprolium was significantly better than both extracts ($P < 0.05$). It is concluded that both AE and EE of *M. oleifera* could serve as safe and cheaper alternative to amprolium especially in rural areas where synthetic drugs are lacking or in farms practicing organic poultry farming.

Keywords: *Moringa oleifera*, seed, Supplementation, *Eimeria tenella*, broiler birds

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Introduction

Coccidiosis due to *Eimeria* species is an important economic disease affecting poultry birds worldwide. The genus *Eimeria* includes protozoan parasites belonging to the Phylum Apicomplexa. Various *Eimeria* species are responsible for the disease coccidiosis in vertebrates (Chartier, 2012). More than 1000 species of protozoan parasites have been reported to parasitize vertebrate's intestinal epithelia causing an economically significant loss (Witcombe and Smith, 2014). Infection in poultry occur when birds ingest feeds contaminated with sporulated oocysts. The disease is characterized by bloody diarrhea, reduced feed intake, loss of weight and sometimes death (Gilberts *et al.*, 2011). In Nigeria, *Eimeria tenella*, *E. acervulina*, *E. necatrix*, *E. bruneti*, *E. mitis*, *E. praecox* and *E. bruneti* are reported to be the major etiological agents of coccidiosis in poultry (Jatau *et al.*, 2012; Mohammed and Sunday, 2015). Poultry coccidiosis is a key stumbling block to successful commercial and backyard poultry farming worldwide due to its associated high mortality rates and massive economic losses.

Control of coccidiosis depends on the use of chemoprophylaxis and immunoprophylaxis such as sulphonamides and pyrimidine derivatives (Oladoja and Olusanya, 2007). The widespread use of these synthetic drugs has resulted in the emergence of resistance strains of the parasites (Witcombe and Smith, 2014). In addition to the issue of drug-resistance and potential hazardous effects of most anticoccidial, most commercial and backyard poultry farmers in developing countries are rejecting the use of synthetic anticoccidial medication due to high cost of drugs and associated challenges especially where organic poultry farming is practiced, prompting the quest for alternate coccidiosis control strategy with the use of natural products from plant sources and natural feed additives derived from plants which are cheaper, safer, healthier and less hazardous to humans and animals (Peek and Landman, 2011).

Moringa oleifera Lam (Moringaceae) is one of the most extensively grown and valuable medicinal plants in Northern Nigeria with studies showing that every portion of *Moringa* species possess useful characteristics that can be of benefit to man (Mishra *et al.*, 2011). *Moringa oleifera* is reported to be rich in mineral elements, vitamins, carotenoids, protein, essential amino acids and phenolics (Ajibade *et al.*, 2013). *Moringa* spp. are used by traditional healers to treat circulatory and cardiac problems in addition to their use as antimalarial, antifungal, antibacterial, and antioxidants (Farooq *et al.*, 2007). Although Ola-Fadunsin and Ademola (2013) reported on the efficacy of acetone leaf extracts of *M. oleifera* on broiler chickens that were naturally infected by *Eimeria* species, to our knowledge no published information is available on the anticoccidial efficacy of *M. oleifera* seed extracts in poultry birds. Hence, this study was carried out to determine the effects of *M. oleifera* seed supplemented diets on *Eimeria tenella* infected broiler birds.

Materials and Methods

Source, Collection and authentication of plant materials

Moringa oleifera seeds were collected from a backyard farm at the Nigerian Defence Academy Ribadu Cantonment, Kaduna, Nigeria. Plant leaves were also collected for authentication in the herbarium of the Department of Biological Sciences, NDA and voucher number: HAD 1902 assigned.

Preparation of Moringa oleifera aqueous seed extracts

Dry *M. oleifera* seeds were sorted, cleaned and ground up to powder using Bajaj blender (Model: Bravo Dlx). Aqueous extract of seeds was prepared according to the method described by Nwobu *et al.* (2010). About 50 g of powdered *Moringa* seeds was weighed in 500 ml beakers and percolated with 300ml distilled water. The beaker was then properly sealed with aluminum foil and allowed to stand for 48 hours to obtain crude aqueous seed extract (AE). The solution was then filtered into a 500 ml conical flask using a funnel and a filter paper. The extracts obtained was concentrated using rotary evaporator at 40°C. Concentrated extracts obtained were refrigerated at 4°C until needed.

Preparation of Moringa oleifera ethanol seed extracts

The ethanol extract was obtained using cold maceration method described by Handa *et al.* (2008). This involves macerating 50 g of the powdered *Moringa* seeds in 300ml of 95% ethanol. The flask was properly sealed with cotton wool wrapped with foil paper and left to stand for 48 hours at room temperature. The solution was then filtered using a filter paper to obtain the extracts. Extract was concentrated in a rotary evaporator at 40°C and refrigerated at 4°C until needed.

Phytochemical screening of M. oleifera seed

Phytochemical screening was carried out on *M. oleifera* seeds extracts using standard procedures (Trease and Evans, 1989, Sofowara, 1990, Odebiyi and Nwobu *et al.* 2010).

Source and isolation of Eimeria tenella oocysts

Field isolates of *E. tenella* were obtained from infected birds in a local broiler farm in Kaduna metropolis, Kaduna State. Infected birds were euthanized and the caecal contents collected and used for oocysts propagation according to the method described by Permin and Hansen (1998). Caecal contents of infected birds were rinsed with tap water into a beaker. The washings of the caecal contents was then transferred to 15ml centrifuge tubes and centrifuged at 3000

rpm for 10 mins. The supernatant was decanted and the resultant pellets were re-suspended in tap water and centrifuged again at 3000 rpm for 10 mins and the supernatant decanted.

Sporulation and propagation of oocysts

Oocysts collected were identified and sporulated in 2.5% Potassium dichromate ($K_2Cr_2O_7$) at room temperature for 4 days (Bowman, 2009). Identification of oocyst was based on oocyst size and number of sporocyst (McDougald and Fitz-Coy, 2008). Sporulated oocysts were washed with 300ml of distilled water and centrifuged at 3000 rpm for 5min to remove $K_2Cr_2O_7$. This process was repeated three times in order to get rid of $K_2Cr_2O_7$ remnant from oocysts. The sporulated *E. tenella* oocysts were then suspended in distilled water and 100000/ml oocysts were orally inoculated into five 16 days old broiler birds each for propagation (Meskerem and Boonkaewwan, 2013). The faeces of inoculated birds were collected and examined daily using a light microscope for the presence of *Eimeria* oocysts. Birds were also monitored for the development of clinical manifestations of coccidiosis. Birds with patent infection were euthanized on day 7 post infection (Pi) for evacuation of caecal content. Oocysts from caecal content were sporulated and stored at 4°C in 2.5% $K_2Cr_2O_7$ until oral administration of experimental birds (Meskerem and Boonkaewwan, 2013).

Source and Management of Experimental Chicks

Seventy (70) day-old broiler chicks, brand products of Agric International Technology and Trade Limited (AGRITED) Ibadan, Oyo State, were purchased through a local dealer in Kaduna. The chicks were transported to a brooder house in birds' dispatch cartons. On arrival at the brooding house, chicks were provided drinking water to which glucose was added to help the chicks overcome transportation stress. The

required brooding temperature (35°C) was provided using 100-watt electric bulb. Infectious bursal and Newcastle Diseases Vaccines (NDV) were administered to all chicks. The chicks were fed commercial broiler standard starter feed (Vital feed) and allowed water *ad libitum*. Prior to purchase of chicks, ethical approval was obtained from NDA Ethical Committee. All experimental chicks were treated humanely.

Experimental design

Seventy 18 days old broiler chicks were randomly divided into seven groups of ten birds per group after brooding. Faecal samples of all birds were collected and checked microscopically for coccidia oocyst. In addition, all birds were examined by a veterinarian for sign of infection.

Experimental infection of chicks

Birds in groups 2-7 were orally infected with 2×10^4 sporulated *E. tenella* oocysts on day 18 after the infective inoculum was adjusted to 1ml with water (Ahmad *et al.*, 2020). Infected birds were monitored daily, and their faeces examined for the presence of *Eimeria* oocysts.

Treatment of experimental chicks

Birds were grouped and treated as follows (Table 1): Group 1: uninfected and untreated (Control); Group 2: infected and fed un-supplemented Vital feed; Group 3: infected and fed Vital feed mixed with amprolium; Group 4: infected and fed Vital feed supplemented with 1g/kg AE; Group 5: infected and fed Vital feed supplemented with 2g/kg AE; Group 6: infected and fed Vital feed supplemented with 1g/kg CESE and Group 7: infected and fed Vital feed supplemented with 2g/kg EE. Treatment of birds commenced on day 6 post infection. Oocysts count, feed intake, live weight and mortality rate in all groups were noted and recorded.

Table 1: Grouping and Treatment Administered to Experimental Birds

Group	Number of birds	Number of sporulated oocyst administered	Treatment/supplement administered
1	10	0	Uninfected Control (Unsupplemented Vital Feed)
2	10	2×10^4	Infected Control (Unsupplemented Vital Feed)
3	10	2×10^4	Vital Feed + 0.6g/kg of amprolium
4	10	2×10^4	Vital Feed + 1g/kg of crude aqueous seed extract
5	10	2×10^4	Vital Feed + 2g/kg of crude aqueous seed extract
6	10	2×10^4	Vital Feed + 1g/kg of crude ethanolic seed extract
7	10	2×10^4	Vital Feed + 2g/kg of crude ethanolic seed extract

Determination of efficacy of treatment

The efficacy of treatments was evaluated on the basis of live body weight, feed intake, oocyst count, mortality rate and caecal lesion score. (Meskerem and Boonkaewwan, 2013). Mean body weight gain, daily feed intake, oocysts output, lesions score and mortality rate were calculated for all groups using the formula described by Cedric *et al.* (2017).

Determination of body weight

The body weight of all experimental birds in each group was weighed before infection and on day 7 post treatment. Using Cedric *et al.* (2017) formula, weight gain per bird and mean weight gain per group were determined as follows:

Weight gain per bird = final weight of bird – initial weight of the same bird.

$$\text{Mean weight gain} = \frac{\text{total weight gain of birds in a group}}{\text{the number of birds in the group}}$$

Determination of Daily Feed Intake (DFI)

Feed intake of all experimental birds was recorded daily during the post infection period. Daily feed intake (DFI) per bird was calculated using the formula of Cedric *et al.* (2017) as follows:

DFI per group =
Initial weight of feed given to a group – Weight of left-over feed in the group.

$$\text{DFI/bird} = \frac{\text{Feed consumed per group}}{\text{Number of birds in the group}}$$

Determination of Eimeria tenella oocyst count

Faecal samples in each cage were collected from randomly selected sites for oocyst count from day 4 through day 12 post infection. Oocyst count was carried out using McMaster chamber method described by Permin and Hansen (1998).

Determination of Mortality Rate

The number of dead broiler birds in each group was recorded daily after experimental infection. Mortality rate in each group was calculated as described by Cedric *et al.*, (2017) as follows:

$$\text{Mortality rate} = \frac{\text{Total No of dead birds in a group}}{\text{Total No of experimental birds in the group}} \times 100$$

Gross Caecal Lesion/Lesion score

Three chickens from each group were sacrificed by cervical dislocation on day 7 post treatment for gross caecal lesion and scoring (Meskerem and Boonkaewwan, 2013). Evaluation of caecal lesion was based on lesion scoring system which employs numerical ranking of gross lesion using a discrete 5-point scale. Rating ranged from 0 to 4, with 0 indicating no lesion, 1 indicating a mild lesion, 2 indicating a moderate lesion, 3 indicating a severe lesion, and 4 indicating an exceedingly severe lesion/death.

Data Analysis

Results were expressed as percentages and means plus or minus standard error (M ± SED). One-way analysis of variance (ANOVA) followed by Tukey's (post hoc) test were used to evaluate statistical significance difference between mean weight and caecal lesion of experimental groups using Statistical Package for Social Sciences (SPSS) version 23. Difference between groups was regarded as significant at P < 0.05.

Results

Phytochemical constituents of the aqueous and ethanol extracts of Moringa oleifera seed

Result of phytochemical screening of *M. oleifera* seeds is presented in Table 2. Alkaloids, glycosides, flavones, saponins, tannins, terpenoids and phenolic compounds were found to be present in both AE and EE of the seed.

Effect of M. oleifera feed supplement on weight of experimental birds

Birds in Group 1 had the highest mean body weight of 656.10 ± 9.72g while birds in Group 2 recorded the least (333.83 ± 29.05g). Birds in Group 3 fed with feed supplemented with amprolium recorded the highest body weight (529.40 ± 8.41g) among infected and treated groups. However, despite the significant difference (P < 0.05) observed between treatment groups and control groups, birds in groups 5 – 7 showed no significant difference (P > 0.05) in mean body weight gain (.480.13 ± 8.41g – 483.50 ± 5.07g) (Table 3).

Effect of M. oleifera feed supplement on Daily Feed Intake

Birds in uninfected control group consumed more feed as reflected by the total feed intake of 1131.1g. This is followed by birds in Groups 3 and 5 with total feed

Effect of M. oleifera supplemented feed on lesion score and mortality

Birds in infected control group recorded the highest lesion score (3.43 ± 0.29) amongst the infected groups while birds in amprolium treated group recorded the least lesion score (0.33 ± 0.33) amongst all infected and treated groups. There was no significant difference ($P > 0.05$) in lesion score amongst the moringa treatment groups.

Birds in infected control group has the highest mortality rate of 40% while the least mortality (10%) was recorded in groups fed with feed supplemented with 1g/kg EE. While other treatment groups recorded mortality of 20% respectively, no mortality was recorded in uninfected control and amprolium treatment groups (Table 5).

intake of 953.7g and 910.4g respectively. However, the total feed intake of birds in Groups 2, 4, 6 and 7 ranged between 886.4g and 891.7g (Table 4).

Effect of M. oleifera supplemented feed on oocysts output

Birds in Group 1 (Uninfected Control) recorded no oocyst of *E. tenella* in their faeces. However, all treatment groups demonstrated significant reduction in total mean oocyst count compared to infected control group ($P < 0.05$). Among the treatment groups, significantly lower oocyst count was recorded in birds fed amprolium supplemented feed (Group 3) ($P < 0.05$). No significant difference in oocyst count was observed in birds of Groups 4, 5, 6, and 7 administered feed supplemented with different concentration of AE or EE of *M. oleifera* ($P > 0.05$) (Table 6)

Table 2: Phytochemical Constituents of Moringa oleifera seed extracts

Phytochemicals	Aqueous extract	Ethanol extract
Alkaloids	+	+
Glycosides	+	+
Flavones	+	+
Saponins	+	+
Steroids	-	-
Terpenoids	+	+
Phenolics	+	+
Tannins	+	+

Note: + Present; - Absent

Table 3: Effect of *M. oleifera* feed supplement on mean body weight gain (MBWG)

Treatment Group	Mean Body Weight Gain \pm SE (g)	P Value
Uninfected Control (1)	656.10 \pm 9.72 ^a	0.000
Infected Control (2)	333.83 \pm 29.05 ^c	
Amprolium (0.6g/kg) (3)	529.40 \pm 1.64 ^b	
Crude Aqueous Seed extract (1g/kg) (4)	494.75 \pm 5.25 ^c	
Crude Aqueous seed extract (2g/kg) (5)	480.13 \pm 8.41 ^d	
Crude Ethanolic seed extract (1g/kg) (6)	481.55 \pm 8.73 ^d	
Crude Ethanolic seed extract (2g/kg) (7)	483.50 \pm 5.07 ^d	

Note: Number in parenthesis represent group. Values with different superscript are significantly different (P < 0.05) using Tukeys' Posthoc Test

Table 4: Effect of *M. oleifera* feed supplement on daily feed intake (DFI)

Treatment Group	Daily Feed Consumed /Bird (g)										Total FI (g)
	1	2	3	4	5	6	7	8	9	10	
UC	79.4	84.3	97.4	114.7	120.2	123.8	125.6	126.9	127.1	131.7	1131.1
IC	76.2	83.5	97.3	105.2	101.8	90.3	89.1	81.7	80.2	81.1	886.4
Ampro (0.6 g/kg)	81.7	91.3	102.2	110.9	100.9	87.2	87.9	89.0	96.3	106.3	953.7
AE 1 g/kg	76.8	89.7	95.6	106.7	97.9	89.2	81.6	82.2	83.4	89.1	892.2
AE 2 g/kg	78.3	87.7	94.7	111.4	101.2	91.8	84.6	83.9	85.2	91.6	910.4
EE 1 g/kg	78.9	87.9	96.5	107.4	91.9	87.6	81.9	81.2	84.9	88.3	886.5
EE 2 g/kg	79.8	86.6	94.2	110.6	96.3	88.3	80.8	81.0	83.7	90.4	891.7

Note: UC- Uninfected control, IC- Infected control, Ampro- Amprolium, AE- aqueous seed extract, EE- ethanol seed extract

Table 5: Effect of *M. oleifera* supplemented feed on lesion Score and mortality.

Treatment Group	Lesion score distribution (%)					Mean Lesion Score ± SE	Mortality Rate (%)
	0	+1	+2	+3	+4		
UC	100	0	0	0	0	0.00 ± 0.00 ^a	0
IC	0	0	14	29	57	3.43 ± 0.29 ^b	40
Ampro (0.6 g/kg)	67	33	0	0	0	0.33 ± 0.33 ^c	0
AE (1 g/kg)	0	40	20	0	40	2.40 ± 0.67 ^d	20
AE (2 g/kg)	0	20	40	0	40	2.60 ± 0.60 ^d	20
EE (1 g/kg)	0	25	50	0	25	2.25 ± 0.62 ^d	10
EE (2 g/kg)	0	40	20	0	40	2.40 ± 0.67 ^d	20

P = 0.000

Note: Number in parenthesis represent group. Values with different superscript are significantly different (p <0.05) using Tukeys' Posthoc Test;

Table 6: Effect of *M. oleifera* supplemented feed on daily oocysts output.

Treatment Group	Daily Oocyst Shed Per Gramm of Feaces (OPG) x10 ²										Total OPG/bird
	4	5	6	7	8	9	10	11	12		
UC	0	0	0	0	0	0	0	0	0	0	0
IC	0	3	63	181	217	166	94	83	46	853	
Ampro (0.6 g/kg)	0	3	61	92	26	11	2	1	0.5	196.5	
AE 1 g/kg	0	8	79	180	73	24	11	7	5	387	
AE 2 g/kg	0	4	67	171	48	19	9	8	5	331	
EE 1 g/kg	0	5	69	180	56	21	13	9	4	357	
EE 2 g/kg	0	5	62	173	41	17	10	7	3	318	

Note: Day 4 – 6: Pre-treatment days, Day 7 – 12: Post treatment days

Discussion

Moringa oleifera plant has been found to possess various secondary metabolites such as saponins, tannins, terpenes, alkaloids, flavonoids, carbohydrates, and cardiac glycosides (Ajibade *et al.* 2013). These phytochemical compounds have potentials in the treatment of many ailments in humans and animals (Fahey, 2005; Aremu *et al.*, 2012). Flavonoids have free radical scavenging activities by preventing oxidative cell damage, as well as numerous therapeutic actions against the oxidative damage induced by many disease processes (Ajibade *et al.*, 2013).

The body weight gain in birds fed with the extracts-supplemented feed could be attributed to the phytochemicals found in the seed. Al-Shaibani *et al.* (2020) in a related work reported higher body weight gain in *Eimeria tenella* infected birds medicated with *Allium sativa*, *Punica granatum* and synthetic drugs than control infected and unsupplemented birds. The authors concluded that the low weight gain recorded among the infected control birds fed unsupplemented feed could be as a result of the damaging effect of *Eimeria* parasite on the epithelial lining which leads to anorexia and poor feed uptake. Arlett *et al.* (2019) also recorded the lowest mean body weight in infected untreated chicken while working to determine the anticoccidial effects of *Ageratum conyzoides* and *Vernonia amygdalina* leaves extracts on broiler chickens.

Similarly, the difference in the mean amount of feed consumed by birds in infected and treated groups compared to birds in uninfected control group could be attributed to the proliferation of *Eimeria tenella* in the intestinal tract of the birds leading to tissue damage and interruption feeding. McDougald and Fitz-Coy (2008) stressed that *Eimeria* species proliferate in the intestinal tract and cause tissue damage, resulting in the interruption of feeding, digestive processes, nutritional absorption, dehydration, blood loss, loss of skin pigmentation and increased susceptibility to other disease pathogens. However, the reduction in oocyst output exhibited by birds fed with feed supplemented with *M. oleifera* seed extracts could be due to coccidiostatic effects of some of the phytochemicals present in *M. oleifera*. Some of these phytochemicals are reported to inhibit oocysts development and release in faeces of *E. tenella* infected birds. Ola-Fadunsin and Ademola (2013) also reported the inhibition of oocyst output as well as faecal score in broiler birds naturally infected with *Eimeria* species and treated with acetone extract of *M. oleifera*. Similarly, significant reduction of oocyst count in faeces was observed by Kaboutari *et al.* (2014) when broilers infected with *E. tenella* were treated with

granulated extract of *Artemisia sieberi*. Although AE and EE of *M. oleifera* may not have therapeutic effects on *E. tenella* as reflected by mortality rate in birds treated with different concentration of the extracts, the plant could be useful in controlling the transmission of the parasite by reducing significantly the level of contamination of feeds and the environment which serves as source of acquiring infection by birds.

Conclusion

In conclusion, the aqueous and ethanol extracts of *M. oleifera* seeds contain phytochemicals that could have some coccidiostatic effect on *E. tenella* in infected birds. It lacked therapeutic effect against intestinal damages associated with coccidiosis. The extracts of *M. oleifera* seeds could be used as food supplement to reduce oocyst output of infected birds, environmental contamination and spread of the coccidiosis among birds.

Conflicts of Interest

There are no conflicts of interest.

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