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Protective Effects of *Moringa stenopetala* leaf supplemented Diets on *Eimeria tenella* Infected Broiler Chickens in Debre Zeit, Central, Ethiopia

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ABSTRACT

Coccidiosis is an intestinal infection caused by intracellular protozoan parasites belonging to several different species of *Eimeria*. Coccidiosis seriously impairs the growth and feed utilization of chickens. *Moringa* leaf is an excellent source of a wide spectrum of dietary antioxidants. It has been reported that antioxidant-rich plant extracts have potential benefits in treating coccidial infections. *Moringa stenopetala* (Moringaceae) has been used as a traditional medicine in some areas of Ethiopia. Leaf powder and extracts of *M. stenopetala* were evaluated for their anticoccidial activity against *Eimeria tenella* infection in broiler chickens. Following oral infection with 12×10^4 sporulated *E. tenella* oocysts, broiler chickens were assigned to five treatments; each group was assigned to one of the five treatments: infected/unsupplemented (control), infected/supplemented (with leaf powder, extract or amprolium) and a control check of noninfected/unsupplemented. The effects of *M. stenopetala* on *E. tenella* infection were assessed by four parameters: survival rate, body weight gain, oocyst count and cecal lesion score. Challenged chickens fed a diet supplemented with *M. stenopetala* as either dry leaf powder or in extract demonstrated a significantly higher reduction in oocyst counts ($P < 0.0001$) than those fed the control or unsupplemented diets. Infected chickens fed the diet supplemented with *M. stenopetala* dry leaf powder demonstrated significantly increased body weight gain ($P < 0.0001$) and reduced cecal lesions ($P < 0.0001$) than those fed the diet supplemented with *M. stenopetala* leaf extract or the control diet. These results suggest that *M. stenopetala* has a protective effect against *E. tenella* infection in chickens. However, *Moringa*-supplemented diets did not reduce mortality.

Keywords: chicken, *Eimeria tenella*, *Moringa stenopetala*, coccidiosis

INTRODUCTION

Coccidiosis is an intestinal infection caused by intracellular protozoan parasites belonging to several different species of *Eimeria*. Infection with coccidia parasites seriously impairs the growth and feed utilization of chickens and costs the USA poultry industry more than USD 1.5 billion in annual losses (Yun *et al.*, 2000). *E. tenella* is the best known of poultry coccidia. This species inhabits the ceca (rarely adjacent intestinal tissues) causing hemorrhage and inflammation of the ceca. It is known as cecal or "bloody" coccidiosis. It can be recognized by the accumulation of blood in the ceca and by bloody droppings (McDougald and Fitz-Coy, 2008). In Ethiopia, poultry coccidiosis

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is endemic in various parts of the country and affects mainly young growing birds; for instance; the prevalence of clinical coccidiosis reported from three commercial broiler farms of Debre Zeit, central Ethiopia was 37.5, 43.33 and 50%, and the species of *Eimeria* identified were *E. acervulina, E. maxima, E. necatrix* and *E. tenella* (Mersha *et al.,* 2009).

Conventional disease-control strategies rely heavily on chemoprophylaxis, which is a tremendous cost to the industry. Existing vaccines consist of live, virulent or attenuated *Eimeria* strains with limited protection against this pathogen. The continual emergence of drug-resistant strains of *Eimeria*, combined with the increasing regulations and a rejection on the use of anticoccidial drugs in commercial poultry production, urges the need for novel approaches and alternative control strategies (Dalloul and Lillehoj, 2005).

It has been reported that antioxidant-rich plant extracts have potential benefits in treating coccidial infections (Allen *et al.*, 1997; Naidoo *et al.,* 2008). The nutrient contents of 3-year-old *M. stenopetala* leaf originating from Ethiopia and Kenya per 100 g fresh weight were found to contain dry matter (24%), protein (5.8 g), iron (5.4 mg), calcium (711 mg) and the concentrations of natural antioxidants (total phenolics and antioxidant vitamins A, C and E) on a dry weight basis were 94, 0.9, 88 and 1.6 $\mu mol.g^{-1}$ for phenolics, β-carotene, ascorbate and α-tocopherol, respectively (Yang *et al.,* 2006). The nutrient composition of raw *M. stenopetala* leaf per 100 g on a dry weight basis was reported as: energy, 295.4 kcal; protein, 9.0%; fat, 5.8%; carbohydrate, 518%; crude fiber, 20.8%; Ca, 793 mg; P, 65.6 mg; Zn, 0.53 mg; Fe, 3.8 mg; β-carotene, 160 µg; and ascorbic acid, 28 mg (Abuye *et al.,* 2003). The amount of short chain fatty acid in *M. stenopetala* leaf was 103 mmol (Melesse, 2012). Nibret and Wink (2010) reported that 100 g of essential oil derived from *M. stenopetala* seeds, contained benzyl isothiocyanate (54.30%), isobutyl isothiocyanate (16.37%), palmitic acid (14.57%) and oleic acid (up to 8.13%).

Moringa is a small genus belonging to the family Moringaceae; the genus comprises 13 species of trees and shrubs distributed in Africa and Asia (Padayachee and Baijnath, 2012). *Moringa oleifera* or the Lam tree is a native of India, occurring wild in the sub-Himalayan regions of Northern India, and is now grown worldwide in the tropics and subtropics (Rajangam *et al.,* 2001). Moringa species have a broad variety of uses in medicine, food, cosmetics and oil production; various parts possess many medicinal uses, pharmacological activities and provide sources of numerous medicinal compounds (Padayachee and Baijnath, 2012). *Moringa stenopetala* (Moringaceae) is endemic to East Africa, where it occurs in northern Kenya and Ethiopia (Bosch, 2004). *Moringa stenopetala* in tree form is known as *aleko* or *shiferaw* among local communities in Ethiopia and commonly grows to 6–10 m. The tree is cultivated for leaves that are boiled and eaten like cabbage. The people use the tree not only for food but also as a medicine (Verdcourt, 2000; Mekonnen, 2005). It is used traditionally for the treatment of different diseases. The Turkana people of northern Kenya make an infusion of the leaf, which is used as a remedy against leprosy. In the Konso area of Ethiopia the smoke of burning roots is used as a treatment for epilepsy and the leaves of certain *M. stenopetala* trees are renowned for their effectiveness against diarrhoea. In the Negelle and Wolayeta Sodo areas of Ethiopia, the leaves and roots are used as a cure for malaria, stomach problems and diabetes. The leaves are also used to treat hypertension, retained placenta, asthma, colds, as an anthelmintic, to induce vomiting and to promote wound healing (Bosch, 2004). The extract of *M. stenopetala* leaf showed an antidiabetic effect in normoglycemic and alloxan-induced diabetic mice (Nardos *et al.*, 2011) and an antispasmodic property on uterus strips of guinea-pigs and mice (Mekonnen, 1999). The essential oil of *M. stenopetala* seeds and its

main compound, benzyl isothiocyanate, showed the most potent trypanocidal activities (Nibret and Wink, 2010). Both the dried leaf and the fresh root extracts of *M. stenopetala* showed activity against *Trypanosoma brucei* (Mekonnen *et al.*, 1999). However, the anticoccidial activity of *M. stenopetala* leaf was not investigated. The current study aimed to evaluate the anticoccidial effects of *M. stenopetala* leaf powder and its extract against cecal coccidiosis in broilers.

MATERIALS AND METHODS

Experimental birds

A sample of 150 one day-old broiler chicks was purchased from Alema (a private commercial broiler farm), Debre Zeit, Ethiopia. All experimental chicks were vaccinated against Newcastle and infectious bursal diseases. Chicks were placed in a brooder house for 18 d. Feed and water was supplemented *ad libitum*. The starter feed was formulated using maize, wheat bran, soybean, noug cake (*Guizotia abysinica*), limestone, vitamin premix and salt. Feed was supplemented without anticoccidial drugs.

Collection and preparation of *M. stenopetala* leaf powder

Fresh *M. stenopetala* leaves were collected during September and November 2011 from Yeki woreda, Teppi coffee farm, south western Ethiopia. The leaves were collected from 7-year-old trees and were dried under shade to avoid nutrient loss associated with exposure to direct sunlight. Drying was allowed for 15 d with a temperature and humidity range of 15–34 ^{o}C and 60–70%, respectively. Leaves were stored for 2 wk until the day before starting the experiment, when leaves were powdered using a mortar and pestle.

Ethanol extract preparation using hot continuous Soxhlet extraction method

Samples of 500 g of *M. stenopetala* leaf powder were each placed in a thimble made of filter paper and inserted into the wide central tube of the extractor of the Soxhlet apparatus. The solvent (ethanol) was placed in the flask and heated at 78 ^{o}C and its vapors condensed in a reflux condenser. The condensed extractant dripped into the thimble containing the crude drug, which was extracted by contact. When the level of the liquid in chamber had risen to the top of siphon tube, the liquid contents of the chamber drained off into the flask. This process was continuous and was carried out until a drop of the solvent from the siphon tube did not leave any residue when evaporated (Handa, 2008). One mL aliquots of the filtrate were taken and concentrated. The concentration of *Moringa* solution was 50 mg.mL^{-1} (23%). The extract was stored in a refrigerator for 1 wk until used and was mixed with feed before use.

Isolation and propagation of *Eimeria* oocysts

Eimeria oocysts used in this study were isolated from chickens with clinical signs of cecal or "bloody" coccidiosis. *E. tenella* oocysts were identified by a combination of oocyst size, location in the gut, appearance of the lesions, and schizonts size (McDougald and Fitz-Coy, 2008). Following evisceration at *post mortem*, the cecal contents were washed into a beaker using tap water and oocysts were isolated using a flotation procedure (Permin and Hansen, 1998). Oocysts were sporulated within 72 h (Bowman, 2009). The sporulated oocysts were orally inoculated in two chickens for oocyst multiplication. Chickens were monitored daily for the development of clinical coccidiosis and the presence of *Eimeria* oocysts in their feces. The sporulated *Eimeria* oocysts were obtained as described earlier. Sporulated oocysts were suspended in 2% $K_2Cr_2O_7$ solution and refrigerated at 4 ^{o}C until oral administration. The $K_2Cr_2O_7$ solution was removed through centrifugation and the sporulated *E. tenella* oocysts were suspended in distilled water at the time of oral administration.

Experimental treatments

The experimental treatments were carried out using a completely randomized design. One hundred fifty chickens were randomly allotted into five treatments (n = 30), with three replications per treatment for a period of 2 wk. The experimental study was conducted at the Debre Zeit agricultural research center poultry farm. Treatments were applied from day 18 of hatching. The chicks were supplemented with the different treatments throughout the experiment for a period of 2 wk. At age 18 d, chickens in treatments 2, 3, 4 and 5 were orally challenged with 12×10^4 sporulated *E. tenella* oocysts in 1 mL of distilled water suspension using a calibrated syringe. Chickens in the first treatment were not challenged. Two days after infection, the chickens in treatments 3, 4 and 5 were respectively supplemented with 0.6 $g.L^{-1}$ of amprolium in drinking water, 1.5 $g.kg^{-1}$ of *M. stenopetala* leaf powder and 1.5 $g.kg^{-1}$ of *M. stenopetala* leaf powder extract in feed, while the chickens in the first (control check) and second (control) treatments were not supplemented (Table 1). Chickens were monitored daily for the presence of clinical signs.

Experimental parameters and data collection

The efficacy of treatments was evaluated on the basis of survival rate, body weight gain (BWG), oocyst count and cecal lesion score. The number of dead chickens was recorded daily until day 7 post challenge. The body weight of all experimental chickens in each group was weighed twice—on day 18 (before challenge) and day 7 post challenge. Feed intake of all experimental chickens in each group was weighed daily during the period of day 18 to day 7 post challenge. Fecal samples from all experimental groups were collected and checked before challenge; no oocysts were detected. Fecal samples in each cage were collected from randomly selected sites and the oocyst count per gram of feces (OPG) was calculated using the technique described by Permin and Hansen (1998) and recorded during the period of day 4 to day 12 post infection. On day 7 after challenge, three randomly selected chickens in each group were euthanized by cervical dislocation for cecal lesion scoring according to the method of Johnson and Reid (1970). The scoring scale ranged from 0 to +4, where 0 = no lesion, 1 = mild lesion, 2 = moderate lesion, 3 = severe lesion and 4 = extremely severe lesion/death.

Data analysis

The data were analyzed using the analysis of variance procedure of the SAS statistical software package (SAS, 2003). Tukey's post hoc test was applied to compare the means of BWG, feed intake, mortality and fecal oocyst count between treatments. Cecal lesion scores were analyzed using general linear models and the means of cecal lesion scores between treatments were compared using least square means. The difference between treatments was considered

Table 1 Experimental treatment in five groups, (30 chickens each), to evaluate the effect of *M. stenopetala* on coccidiosis caused by *E. tenella.*

Group	Number of chicks	Treatment	Oocysts for challenge with 12×10^4 *E. tenella*
1	30	Control check (noninfected and unsupplemented)	–
2	30	Control (infected usupplemented)	+
3	30	Feed with 1.5 $g.kg^{-1}$ of leaf powder	+
4	30	Feed with 1500 $mg.kg^{-1}$ of extract	+
5	30	0.6g of amprolium.L^{-1} in drinking water	+

– = No infection with oocysts, + = Infection with oocysts.

significant at the ($P < 0.05$) level.

RESULTS

Oocysts per gram, mortality and lesion score

Challenged chickens fed a diet supplemented with either dry leaf powder or leaf extract showed significantly reduced ocysts counts of 43 ± 3.46 and 25 ± 5.68 ($P < 0.0001$), respectively compared with those fed unsupplemented diets 939 ± 53.5. However, *Moringa* leaf supplemented diets did not reduce the mortality in *E. tenella*-infected chickens (Table 2).

E. tenella-challenged chickens fed the diet supplemented with *M. stenopetala* dry leaf powder demonstrated a significantly reduced cecal lesion score of 3.44 ± 0.14 ($P < 0.0001$) compared to those fed diets that were extract supplemented (3.56 ± 0.14) and unsupplemented (3.89 ± 0.14), respectively, as shown in Table 3.

Average daily gain and average daily feed intake

The average daily gain (ADG) of *E. tenella*-infected chickens fed the diet supplemented with *M. stenopetala* dry leaf powder was significantly greater 5.59 ± 0.59 g ($P < 0.0001$) than in those fed the extract supplemented and unsupplemented diets (3.66 ± 1.33 and 2.19 ± 1.25 g), respectively (Table 4).

Table 2 Oocysts count per gram of feces (OPG) and mortality rate in *E. tenella* challenged chickens fed diet supplemented either with or without *Moringa stenopetala*, or with or without amprolium.

Treatment	OPG (× 100)	Mortality (%)
Control check (nonifected and unsupplemented)	0.0 ± 0.0^{b}	00 ± 00^{b}
Control (infected unsupplemented)	939 ± 54.5^{a}	30 ± 20^{a}
Infected + amprolium	30.0 ± 5.0^{b}	00 ± 00^{b}
Infected + *M. stenopetala* leaf powder	43.0 ± 3.5^{b}	23 ± 12^{ab}
Infected + *M. stenopetala* leaf extract	24.7 ± 5.7^{b}	23 ± 15^{ab}
P-value	0.0001	0.011

^{a,b} = Means in a column with different lowercase superscript letters vary significantly ($P < 0.05$).

Table 3 Lesion score results in *E. tenella*-challenged chickens fed diet supplemented either with or without *Moringa stenopetala*, or with or without amprolium.

Treatment	LSM lesion score ± SE
Control check (noninfected and unsupplemented)	00.0 ± 0.14^{c}
Control (infected unsupplemented)	3.89 ± 0.14^{a}
Infected + amprolium	3.22 ± 0.14^{b}
Infected + *M. stenopetala* leaf powder	3.44 ± 0.14^{b}
Infected + *M. stenopetala* leaf extract	3.56 ± 0.14^{ab}
P-value	0.0001

^{a,b,c} = Means in a column with different lowercase superscript letters vary significantly ($P < 0.05$).

LS = Least squares means, SE = standared error

Table 4 Average daily gain (ADG) and average daily feed intake (ADFI) of *E. tenella*-challenged chickens fed diet supplemented either with or without *Moringa stenopetala*, or with or without amprolium.

Treatment	ADG (g per chick)	ADFI (g per chick)
Control check (noninfected and unsupplemented)	16.18 ± 3.08[a]	47.73 ± 4.04[a]
Control (infected unsupplemented)	2.19 ± 1.25[c]	36.73 ± 2.99 [a]
Infected + amprolium	9.38 ± 0.36[b]	42.02 ± 4.98[a]
Infected + *M. stenopetala* leaf powder	5.59 ± 0.59[b]	38.46 ± 5.75[a]
Infected + *M. stenopetala* leaf extract	3.66 ± 1.33[c]	39.45 ± 1.51[a]
P-value	0.0001	0.085

[a,b,c] = Means in a column with different lowecase superscript letters vary significantly ($P < 0.05$).

DISCUSSION

The assay in this study was used to evaluate the anticoccidial effects of *M. stenopetala* leaf against *E. tenella* infection in broilers. The results demonstrated that a diet supplemented with either *M. stenopetala* leaf powder or leaf extract could significantly reduce the oocyst count of *E. tenella*-infected chickens similarly to those supplemented with amprolium when compared to the control chickens.

A diet supplemented with Moringa leaf powder could reduce the cecal lesion scores of *E. tenella*-infected chickens similarly to those supplemented with amprolium, whereas in contrast, chickens fed a diet either supplemented with leaf extract or unsupplemented showed increased cecal lesions scores.

Previous studies have indicated that diets with antioxidant rich plant have an anticoccidial activities (Allen *et al*., 1997; Naidoo *et al.,* 2008). *Moringa* leaf is an excellent source of a wide spectrum of dietary antioxidants, such as phenolics and vitamins A, C and E that is, of β-carotene, ascorbate and α-tocopherol, respectively, (Abuye *et al.*, 2003; Yang *et al.,* 2006), and short chain fatty acids (Melesse, 2012). The effects of Moringa might be associated with the dietary antioxidants because antioxidants are capable of scavenging free radicals (Sies, 1997).

Sies (1997) discussed the process of oxidant uptake and the effect of antioxidants. Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. Intestinal mucosal cells are exposed to a variety of reactive intermediates and xenobiotics and the rate of accumulation of products of oxidative damage in these cells is high. Intake of plant-based exogenous antioxidant capable of scavenging free radicals may help to limit the oxidative stress and prevent the damage caused by free radicals. Antioxidant defense involves several strategies, both enzymatic and non-enzymatic. In the lipid phase, tocopherols and carotenes as well as oxy-carotenoids are of' interest, as are vitamin A and ubiquinols (Sies, 1997).

Moringa stenopetala showed anticoccidial effect similar to *Artemisia annua*. Brisibe *et al.* (2008) and Dragan *et al.* (2010), reported significantly reduced fecal oocysts and lesion scores in chickens infected with *E. tenella* and treated with *Artemisia annua* (an antioxidant rich plant), when compared to the *E. tenella*-infected and the nontreated control groups.

It was also reported that immunized chickens fed diets supplemented with 25 ppm selenium or 100 IU vitamin E.kg^{-1} could increase

the body weight gain and feed intake compared to chickens fed an unsupplemented diet after a challenge with 150,000 oocysts of *Eimeria tenella,* while dietary supplementation with selenium or vitamin E could also reduce the mortality and increase the body weight gain of nonimmunized chickens infected with *E. tenella* (Colnago *et al.,* 1984). Diets supplemented with 65 $mg.kg^{-1}$ levels of vitamin E could positively affect performance and improve humoral immune response, mainly in birds vaccinated against coccidiosis (da Silva *et al.,* 2009). Melesse *et al*. (2011) also indicated that chickens fed *M. stenopetala* leaf meal (MSLM) diets showed higher average weight gain than those fed the control diet. The dry matter and crude protein intake and average weight gain of chicks fed with MSLM diets increased with an increasing level of MSLM. *Moringa stenopetala* showed similar results to those reported by previous authors as *M. stenopetala* leaf is rich not only in dietary antioxidants but also in energy, protein, fat, carbohydrate and minerals (Abuye *et al.*, 2003; Yang *et al.,* 2006). These may have contributed to the improvement in body weight gain. Chickens fed a diet supplemented with *M. stenopetala* leaf powder showed significantly higher average weight gain than those fed the unsupplemented diet. However, the supplement did not reduce the mortality in *E. tenella*-infected chickens.

Chickens fed *M. stenopetala* leaf powder extract did not improve their average weight gain nor did the leaf powder extract reduce the lesion score of *E. tenella*-infected chickens. This could have been due to the hot extraction process. High temperature may affect the antioxidant effects of *M. stenopetala* leaf extract. Abuye *et al*. (2003), showed that the ascorbic acid content in cooked *M. stenopetala* leaf decreased by about ten-fold when compared to that in raw leaf, probably due to oxidation of the vitamin C in the leaf during cooking; however, the carotene content decreased by only 11%.

The lack of an improvement in the ADG and no reduction in either the lesion score of chickens fed a diet including *M. stenopetala* leaf extract or in the mortality in chickens fed a diet supplemented with *Moringa* could have been due to the duration of the *M. stenopetala* supplementation. In this study, chickens were fed the diets supplemented with *Moringa* leaf starting 2 days after challenge until 7 days post challenge. It has been reported that the regeneration of the epithelium and glands may be complete within 10 days in light coccidian infections (McDougald and Fitz-Coy, 2008); however, the epithelium may never completely recover in severe infections. In addition, the lost muscularis mucosa is not replaced, and the sub mucosa becomes densely fibrosed (McDougald and Fitz-Coy, 2008). Previous studies by Allen *et al*. (1997) indicated that dried leaf of *A. annua* fed over a period of 3 wk at a level of 5% could significantly reduce lesions; the supplementation of pure compounds of artemisinin over a period of 3 wk also could promote weight gain in chickens infected with *E. tenella*. Its activity may depend on the length of time it is administered before a challenge (Allen *et al*., 1997).

In conclusion, supplementation of the diet with *M. stenopetala* dry leaf powder has a protective effect against *E. tenella* infection. It could improve BWG and reduce cecal lesions in *E. tenella*-infected chickens. Diets supplemented with either *M. stenopetala* dry leaf powder or leaf extract were also effective in reducing the numbers of oocysts shed; however, diets supplemented with Moringa leaf could not reduce mortality. The diets supplemented with Moringa leaf extract did not improve BWG and did not reduce the cecal lesion score of challenged chickens. This might have been due to the extraction process and the short period of time the Moringa supplementation was used. These results needs to be further investigated with cold extract and with a prolonged supplementation time of the diet supplemented with Moringa leaf.

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