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# In-Vivo Anticoccidial Activity of Crude Leaf Powder of *Psidium guajava* in Broiler Chickens

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Original Research Article

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**Abstract:** Coccidiosis caused by *Eimeria* species is one of the major factors militating against profitable poultry production globally because of its serous economic consequences. Continuous and misuse of synthetic anticoccidial drugs over the years had led to the emergence of resistant *Eimeria* strains necessitating the search of alternative, effective safe and locally available coccidiostat. Thus, the anticoccidial activity of crude leaf powder of *Psidium guajava* was tested against *Eimeria tenella* in 120 broiler chicks infected through the oro-pharygeal route with sporulated oocysts of the parasite. The experimental birds were allotted to four groups designated 1 to 4 each having 3 replicates with 10 birds per replicate in a completely randomize fashion. Groups 1 and 2 were fed diet containing 100g and 50g of the test plant per every Kg of feed respectively. Group 3 was treated with a preventive dose of Amprolium (0.3g per liter of drinking water) and group 4 was left untreated. The test material incorporated at 100g per Kg of feed compared appreciably with the Amprolium in suppressing oocysts excretion, alleviating caecal damage, reducing severity of diarrhoea, minimizing blood loss, morbidity and mortality of chicks. The 100g of the test substance was more effective against the parasite than the 50g but both the levels of inclusion had some negative effect on feed intake and weight gain. Purification of the test substance could enhance its efficacy thereby comparing favorably with the drug. Nevertheless, the evaluated plant is still recommended for prophylactic treatment of coccidiosis. The unpalatable taste of the test material which probably reduced the intake of the experimental diet with correspondingly decreased weight gain could be substantially improved through some appropriate measures one of which could be addition of molasses
**Keywords:** Anticoccidial activity, *Psidium guajava*, *Eimeria tenella*, Broiler chicks.

## INTRODUCTION

Poultry farming is the most commercialized of all the agricultural sub-sector in Nigeria and contributes about 25% of the agricultural Gross Domestic Product [1]. However, the poultry industry as is the case all over the world is faced with health challenges, one the major being coccidiosis caused by Eimeria species. This parasitic disease inflicts great economic loss globally [2-4] such that there is a dire need for effective remedial measures.

The control of coccidiosis has for several decades been through the use of synthetic anticoccidial drugs like Amprolium. Continuous and misuses of the drugs over the years had led to the emergence of resistant *Eimeria* strains which had rendered many of the drugs ineffective [5]. The high cost of the current anticoccidial agents, their limited availability especially in remote areas coupled with consumer pressure to have drug free poultry products based on health concern have also limited the application of the conventional anticoccidial medications in managing the disease [6]. Consequently the search for alternative effective, safer and locally available anticoccidial agents have become imperative.

## MATERIALS AND METHODS

### Collection and Preparation of Leaf Powder of *P. guajava*

The leaves of *P. guajava* were collected from Bauchi town. They were then washed in salt solution to remove possible microbial contaminants and rinsed in clean water after which they were spread to dry under shade at room temperature. The dried leaves were crushed into coarse powder using an electric grinder, dried again under shade at room temperature and then ground into fine powder. The resultant fine powder was then stored in nylon bags until required for use.

### Isolation and sporulation of *Eimeria tenella* oocysts

*Eimeria tenella* oocysts were obtained from caecal contents of broiler chicks with lesions consistent with those of cecal coccidiosis after postmortem examination. The oocysts present in the cecal contents

were isolated sporulated and estimated as described by [7].

**Experimental birds**

A total of 120 day old broiler chicks were purchased from Zartech Nigeria Limited Ibadan, Oyo State, and Southwestern Nigeria. These were then brooded for 3 weeks on wire floored cages under hygienic conditions and subjected to routine vaccinations. At 21 days of age, they were transferred to clean, dry and disinfected deep litter cages that were vacant for over 6 months. The experimental birds were given commercial broiler ration and clean drinking water *ad- libitum* throughout the experimental period.

**Experimental Procedure**

All the chicks were infected through the oro-pharyngeal route at the age of 21 days with sporulated oocysts suspension of *E. tenella*. Each bird received 2ml of the suspension which after standardization was found to contain approximately 44,000 oocysts. They were then allotted to 4 groups designated 1 to 4 and each had 3 replicates in a completely randomized design with 10 birds per replicate. Group 1 and 2 were fed diet containing 100g and 50g of the leaf powder of *P.guajava* per kg of the diet respectively. Group 3 were given drinking water containing 0.3g per litre of the drug Amprolium $200^{R}$ (an anticocidial drug PANTEX HOLLAND) in their drinking water. Group 4 was the untreated control. All the treatments were administered for a period of 14 days.

Experimental birds were monitored for mortality and signs of cecal coccidiosis like bloody diarrhea, inappetence and somnolence. Chicks that died were subjected to postmortem examination [8].

Feces of birds in each replicate was scored from the $4^{th}$ to the $10^{th}$ day post-infection on a scale of 0 - 4 [9]. About 2g of the feces was collected daily from 3 different spots in each replicate making a total of 6g from the $5^{th}$ to the $10^{th}$ day post infection and the oocysts content estimated by centrifugal flotation technique [7]. Furthermore, three (3) birds were randomly selected and sacrificed from each replicate on day 7 post inoculation and their ceca examined for lesions, which were scored on a scale of 0 to 4 [10]. In addition, on day 7 and 14 post inoculation, whole blood samples were collected from the wing veins of 3 birds in each replicate and the PCV were determined [11]. The daily feed intake, weekly weight gain and feed conversion ratio of the birds in each treatment group were also evaluated based on standard procedure.

**STATISTICAL ANALYSIS**

The data obtained were analyzed using SPSS version 21and the statistical tools used were one way ANOVA, Kruskal Walli's one way ANOVA and descriptive statistics. The level of significance was set at 95% probability.

**RESULSTS**

Table 1 shows the effects of the crude leaf powder of *Psidium guajava* on mean daily oocysts excretion of birds infected with *Eimeria tenella oocysts*. The total oocysts density excreted for all the treatment groups was highest on day five post infection. Thereafter, the oocysts density declined steadily in all the groups up to day ten when it became lowest in Amprolium treated group followed by 100g *P. guajava* treated group and then 50g *P. guajava* treated group. The infected non treated group excreted the highest oocysts density all through the experimental period.

**Table-1: Effect of crude leaf powder of *Psidium guajava* on mean daily oocysts excretion of birds infected with *Eimeria tenella* oocysts**

| Treatment groups | Mean post infection daily oocyst count per gram of feces | | | | | |
|---|---|---|---|---|---|---|
| | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 |
| 1 (100g *P. guajava*) | 8,350.00 | 5,150.00 | 2,366.66 | 2,444.44 | 1,322.22 | 400.00 |
| 2 (50g *P. guajava*) | 13,450.00 | 10,477.77 | 7,988.88 | 7,950.00 | 1,038.88 | 761.11 |
| 3(Amprolium) | 10,100.00 | 5,122.22 | 1,611.11 | 2,967.77 | 183.33 | 52.22 |
| 4 (infected non-treated) | 117,272.22 | 67,577.77 | 45,700.00 | 53,361.11 | 32,888.88 | 14,055.55 |

The effect of the crude leaf powder of *Psidium guajava* on cecal lesion of birds infected with *Eimeria tenella* oocysts is shown in Table 2. Out of the 9 birds examined for cecal lesions in the group treated with 100g *Psidium gujaava*, 1 (0.11%) had a normal ceca while the remaining 8 showed lesions ranging from mild to moderate. Birds treated with 50g of the test material had cecal lesions ranging from moderate to severe. Majority of birds treated with Amprolium had normal ceca with only 4(44.4%) showing mild lesions. All the birds in the infected non treated group were found to have lesions ranging from severe to very severe. Overall the test substance had significantly alleviated the cecal lesions of the *E. tenella* infected birds ($P<0.05$).

**Table-2: Effect of crude leaf powder of *Psidium gujaava* on cecal lesion of birds infected with *Eimeria tenella* oocysts**

| Treatment groups | No Examined | No. with cecal lesion | | | | |
|---|---|---|---|---|---|---|
| | | Normal (%) | Mild (%) | Moderate(%) | Severe(%) | Very severe(%) |
| 1 (100g *P. guajava*) | 9 | 1 (0.11) | 5 (55.6) | 3 (33.3) | 0 (00.0) | 0 (00.0) |
| 2 (50g *P. guajava*) | 9 | 0 (00.0) | 0 (00.0) | 3 (33.3) | 6 (66.7) | 0 (00.0) |
| 3(Amprolium) | 9 | 5 (55.6) | 4 (44.4) | 0 (00.0) | 0 (00.0) | 0 (00.0) |
| 4 (Infected non-treated ) | 9 | 0 (00.0) | 0 (00.0) | 0 (00.0) | 2 (22.2) | 7 (77.8) |
| Total | 36 | 6 (16.7) | 9 (25.0) | 6(16.7) | 8(22.2) | 7 (19.4) |

P<0.05

The results of the efficacy of the test materials on average daily fecal score of experimental birds are depicted in Table 3. The highest level of fecal abnormality of 2.00 (moderately wet) was observed in the infected non treated group on day 4 post infection. This was followed by the fecal score of 1.44 in the group treated with the 50g of the test plant leaves while a near normal score was seen in the group treated with the 100g of the test substance. The group placed on the Amprolium similarly had almost normal feces on that day. From day 4 post infection the level of fecal abnormality continued to increase on daily basis up to day 7 when it peaked in all the groups thereafter, it started changing to normal when it became almost normal (0.33) and (0.11)in the 100g *P.guajava* treated group and the Amprolium treated group respectively.

**Table-3: Effect of crude leaf powder of *Psidium guajava* on average daily fecal score of birds infected with *Eimeria tenella* oocysts**

| Treatment groups | Mean post infection daily fecal score | | | | | | | Total mean fecal score |
|---|---|---|---|---|---|---|---|---|
| | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | |
| 1 (100g *P. guajava*) | 0.67 | 1.22 | 2.00 | 3.11 | 2.00 | 1.00 | 0.33 | 1.72 |
| 2 (50g *P. guajava*) | 1.44 | 2.33 | 2.67 | 3.78 | 3.33 | 2.00 | 1.11 | 2.48 |
| 3 (Amprolium) | 0.22 | 1.33 | 2.11 | 2.00 | 1.22 | 0.56 | 0.11 | 1.24 |
| 4 (infected non-treated) | 2.00 | 2.67 | 3.56 | 3.89 | 3.67 | 2.56 | 2.00 | 2.96 |

Key: 0 = Normal feces; 1 = Slightly wet; 2 = Moderately wet; 3 = Very wet with blood; 4 = Bloody with absence of normal fecal conistecy

The packed cell volume analysis in response to the various treatments is presented in Table 4. The mean PCV of birds in all the treatment groups fell below normal on day 7 after infection. However, the PCV values of the 100g *P. guajava* treated group and that of the Amprolium treated group did not differ significantly but both differed from those of the other groups (P<0.05). Furthermore, the PCV values appreciated on day 14 post infection when it became normalized in the 100g test material dose as well as in the Amprolium treated group.

**Table-4: Effect of crude leaf powder of *Psidium guajava* on packed cell volume of chickens infected with *Eimeria tenella* oocysts**

| Treatment groups | Normal PCV range 35-55 | Mean PCV Day7 ±SE | Mean PCV Day14 ±SE |
|---|---|---|---|
| 1 (100g *P. guajava*) | | $29.44^{c}$±0.50 | $34.67^{c}$±0.50 |
| 2 (50g *P. guajava*) | | $26.11^{b}$±1.00 | $31.22^{b}$±0.94 |
| 3 (Amprolium) | | $30.89^{c}$±0.40 | $35.89^{c}$±0.63 |
| 4 (infected non-treated) | | $21.67^{a}$±0.83 | $27.11^{a}$±0.92 |

Means with different superscripts within the same column indicate groups that are significantly different from each other (P<0.05)

The data on the activity of the test materials on morbidity and mortality of birds infected with *E. tenella* is given in Table 5. The lowest morbidity was observed in the Amprolium treated group, followed by the the 100g *P. guajava* treated group. Conversely, higher morbidity was recorded in the 50g *P. guajava* treated group and the infected non-treated. Thus, there was a significant difference in morbidity due to the *E. tenella* infection (P<0.05).

Highest mortality was recorded in the infected non treated group. The mortality decreased with increase concentration of the test material with no mortality observed in the Amprolium treated group (P<0.05).

**Table-5: Effect of crude leaf powder of *Psidium guajava* on mortality and morbidity of birds infected with *Eimeria tenella* oocysts**

| Treatment groups | No. of birds/ group | Morbidity(Mean±SE) | Mortality (Mean±SE) |
|---|---|---|---|
| 1 (100g *P. guajava*) | 30 | 13(4.33±0.33$^{b}$) | 1(0.33±0.33$^{a}$) |
| 2 (50g *P. guajava*) | 30 | 22(7.33±0.33$^{c}$) | 2(0.67±0.33$^{a}$) |
| 3 (Amprolium) | 30 | 9(3.00±0.00$^{a}$) | 0(0.00±0.00$^{a}$) |
| 4 (Infected non-treated ) | 30 | 30(10.00±0.58$^{d}$) | 7(2.33±0.33$^{b}$) |

Means with different superscripts within the same column indicate groups that are significantly different from each other (P<0.05)

The influence of treatment on feed intake and feed conversion ratio of experimental chickens is displayed in Table 6. The Amprolium treated group and the infected non treated group had highest and statistically similar feed consumption. On the other hand, the test plant exerted a slight negative effect on the feed consumption which declined with increased inclusion (P<0.05). However, the test material as well as the Amprolium did not have any adverse effect on the feed conversion ratio.

**Table-6: Effect of crude leaf powder of *Psidium guajava* on feed intake and feed conversion ratio of chickens infected with *Eimeria tenella* oocysts**

| Treatment groups | Feed intake (g) | Feed conversion ratio |
|---|---|---|
| 1 (100g *P.guajava*) | 749.02±24.60$^{a}$ | 1.44±0.17$^{c}$ |
| 2 (50g *P.guajava*) | 800.33±11.60$^{b}$ | 1.03±0.02$^{abc}$ |
| 3 (Amprolium) | 903.63±8.85$^{c}$ | 0.82±0.01$^{a}$ |
| 4 (Infected non-treated) | 888.87±7.40$^{c}$ | 1.36±0.27$^{bc}$ |

Means with different superscripts within the same column indicate groups that are significantly different from each other (P<0.05)

Data on weight gain of chickens in the different treatment groups during the experiment is in Table 7. In this regard, chicks in the positive control group gained the highest weight (P<0.05). Though, the weight gains of the birds in the other treatment groups vary numerically, but they were nonetheless statistically similar.

**Table-7: Effect of crude leaf powder of *Psidium guajava* on weight gain of chickens infected with *Eimeria tenella* oocysts**

| Treatment groups | Initial weight (g) | Final weight (g) | Weight gain (g) |
|---|---|---|---|
| 1 (100g *P.guajava*) | 596.67±12.00$^{a}$ | 1150.00±46.19$^{a}$ | 533.33±49.78$^{a}$ |
| 2 (50g *P.guajava*) | 620.00±11.55$^{a}$ | 1390.00±30.55$^{bc}$ | 780.00±17.32$^{bc}$ |
| 5 (Amprolium) | 636.67±14.53$^{a}$ | 1736.67±24.04$^{d}$ | 1100.00±10.00$^{d}$ |
| 6 (Infected non-treated) | 613.33±14.53$^{a}$ | 1310.00±138.56$^{abc}$ | 700.00±124.23$^{abc}$ |

Means with different superscripts within the same column indicate groups that are significantly different from each other (P<0.05)

## DISCUSSION

The efficacy of the test material in reducing the fecal oocysts density of *Eimeria tenella* is interesting. This desirable effect could be attributed to the phytochemical contents of the plant [12]. These workers revealed that plant chemicals like those found in the leaf of the teste plant are effective against protozoa of which *E. tenella* is an example. One of the phytochemicals, saponin, earlier reported to be present in the leave of the plant has been found to bind with the sterol molecules on the cell membrane of parasites and destroy them [13-15]. The effect of the screened plant in reducing the faecal oocyst density is also similar to its activity against other related organisms such as *G. lamblia* [16] *T. gondii* [17], *T. brucei brucei* [18] and *Plasmodium* spp [19, 20]. This underscores its usefulness in the management of protozoan parasitic infection.

The alleviation of caecal lesions exhibited by the treatment plant is impressive. This might be due to its ability to depress oocyst multiplication as earlier observed in this study; it may also be associated with the amelioration of the deleterious effects of the parasitism probably through its antioxidant as well as free radical scavenging properties as observed by earlier workers [21-23]. The observation of attenuation of the cecal lesion in this study is similar to the finding of [17] which revealed that *P. guajava* leave effectively reduced lesions by another coccidian, Toxoplasma.

The reduction of the caecal damage and the partial inhibition of the *E. tenella* oocyst multiplication identified in this study as well as the earlier reported antibacterial activities of the leaves of the test plant appeared to be responsible for the decreased severity of

diarrhoea observed in the treated birds. One of the complications of coccidioisis which contributes to the development of clinical signs and intestinal pathology is secondary infection by enteric bacteria such as *Escherichia coli*, *Salmonella spp* etc [24, 25]. The leaf of the test plant has been shown to inhibit these bacteria [26, 27]. Thus complication arising from secondary infections are eliminated which reduced the bloody faeces.

Furthermore, the effectiveness of the test material in minimizing blood loss is worth noting. This corroborate the report of [28] who showed that guava leaves contain B complex vitamins, Niacin, Folate, Iron, Zinc and Copper all of which contribute to blood formation. Therefore consumption of the test plant will have an additional advantage of being a food supplement that will enhance blood production and resistivity to infection aside from its role in managing coccidiosis.

Reduction in morbidity and mortality of the affected birds is undoubtedly advantageous to the farmer since the threat of losing an entire flock is substantially eliminated. Our result is also in consonance with the report of [29] that guava leaves contain Vitamin C which is known to have immunostimulatory effects leading to resistivity to disease.

The reduced feed intake leading to decreased weight gain observed in the groups treated with the test material could be attributed to the unpalatable taste of the material [30, 31] reported the presence of functional taste buds in the oral cavities of chicken that enable them to detect the tastes of different dietary substances. However, the palatability of the test substance could be improved by incorporation of molasses. By so doing feed improved feed consumption leading to increased weight gain which is the ultimate desire of every broiler farmer could be achieved.

## CONCLUSION

In conclusion, this study demonstrated that crude leaf powder of guava compared appreciably with the drug Amprolium in reducing blood loss, morbidity and mortality, minimizing oocysts density, alleviating caecal damage and decreasing the severity of diarrhoea. However, the test plant showed a slight negative effect in making the feed to be less palatable with a correspondingly low weight gain. Since the test substance is in crude form, there is a high possibility that when it is purified, its efficacy would be enhanced so as to compared favorably with Amprolium. The palatability of the taste could be substantially improved through some measures one of which is addition of molasses to the feed.

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