Effects of Components of Artemisia annua on Coccidia Infections in Chickens

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ABSTRACT
Four experiments were run to test the anticoccidial activity of dried Artemisia annua leaves and several of their chemical constituents for possible use as prophylactic feed additives. When fed over a period of 3 wk at a level of 5%, a dried leaf supplement of A. annua provided significant protection against lesions due to Eimeria tenella but not Eimeria acervulina or Eimeria maxima. When fed over a period of 5 wk at a level of 1% to chicks undergoing immunization with a live vaccine, it provided significant protection in partially immunized chicks against E. acervulina and E. tenella lesions from a dual species challenge infection. It also afforded lower mean lesion scores in challenged chicks immunized over a period of 5 wk. Artemisinin, an antimalarial component of A. annua, was present at a level of 0.034% in the dried leaf preparation. A 5% supplement thus afforded about 17 ppm artemisinin. When the pure compound was fed at that level for a period of 3 wk, it protected weight gains and significantly reduced lesion scores attributable to E. tenella but not E. acervulina. Other components of A. annua, camphor and 1,8-cineole, at 119 ppm also protected weight gains, and reduced E. tenella lesion scores. Camphor reduced E. acervulina lesions. Artemisinin fed for 4 wk at levels of 2, 8.5, and 17 ppm significantly reduced oocyst output from separate E. acervulina and E. tenella infections and a dual species infection. Pure artemisinin thus appears to be effective against at least two coccidia species when used as a feed additive, and its activity may depend, in part, on the length of time it is administered before a challenge infection.

(Key words: coccidiosis, Artemisia annua, artemisinin, anticoccidial, feed additive)

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INTRODUCTION
The increasing resistance of avian coccidia (protozoa) to anticoccidial drugs currently used by the poultry industry has stimulated the search for new methods of control. As part of this effort we have investigated Artemisia annua (annual wormwood) as a potential source of compounds with anticoccidial activity. In China, teas and decoctions of this plant have been used to treat human malaria (also protozoa) for over 2,000 yr (Qinghaosu Antimalarial Coordinating Research Group, 1979). The antimalarial activity in the leaves of this plant was found to be associated with artemisinin, a sesquiterpene lactone possessing an endoperoxide functional group (reviewed by Klayman, 1985). The mode of action of artemisinin has been attributed both to its potential to induce a state of oxidative stress through the free radical cascade generated by the endoperoxide function (Krug-krai and Yuthavong, 1987; Levander et al., 1989; Meshnick et al., 1989), and to the ability of the free radical to alkylate protein (Yang et al., 1994).

Because recent experiments in our laboratory (Allen et al., 1996) have indicated that dietarily induced oxidative stress can be effective in reducing cecal lesions due to Eimeria tenella, we were interested in determining whether whole A. annua leaves would also be active against that and perhaps other avian coccidia species when used as a feed additive, and its activity may depend, in part, on the length of time it is administered before a challenge infection.

MATERIALS AND METHODS

Chickens and Housing
Chickens used were male Sex Sals obtained as day-old chicks. The chicks were either raised in brooders for 2 or 3 wk and then transferred to wire suspended cages (464 cm² per bird) for up to 2 wk, or raised for up to 5 wk in pens (650 cm² per bird) floored with litter to a depth of 5 cm.

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1157

**Parasites**

The parasites used in these experiments: *E. acervulina* 12, *E. tenella* 10 and *E. maxima* 68 were laboratory strains maintained at the facilities of the Parasite Biology and Epidemiology Laboratory by periodic passage through young chickens. All challenge infections were initiated by administering by gavage to each chick a suspension of the requisite number of sporulated oocysts in a volume of 1 mL.

**IMMUCOX®**

IMMUCOX®6 is a live vaccine consisting of a proprietary mixture of oocysts of *E. acervulina, E. tenella, E. maxima,* and *Eimeria necatrix,* and a diluent, and is packaged in quantities to treat 1,000 chicks. Appropriate proportions were prepared so that each chicken received the intended dose of the oocyst mixture in a 1 mL-gavage.

**General Challenge Protocol**

Chick weights were measured just prior to challenge infection, and birds were grouped (Gardiner and Wehr, 1950) so that no significant differences existed between group mean weights. Birds were inoculated by gavage. At 6 d postinoculation (PI) birds were weighed, bled, killed, and lesions in the appropriate regions of the intestine scored (Johnson and Reid, 1970).

**Oocyst Output Determination**

In Experiment 4, droppings from each group (cage) of infected chickens were collected over the 16 h prior to the end of the experiment (approximately 124 to 144 h PI). The total oocysts in each collection was determined from duplicate counts (McMasters chamber, Hodgson, 1970) of duplicate serially diluted aliquots of homogenates (total of four counts per homogenate). The output per chick per group was calculated by dividing the total number of oocysts by the number of chicks per group.

**Experimental Diets**

Regular starter feed was ground to a mash to facilitate mixing of additives. The dried leaf preparation of *A. annua* was mixed directly with the ground feed by weight.

Test compounds (artemisinin, camphor, 1,8-cineole and essential oil), were each dissolved in 20 mL sesame oil, and these solutions individually combined with 200 g ground feed to make premixes. The premixes were then each incorporated into 10 kg of ground feed. Control diets contained the same amount of sesame oil used to dissolve the test compounds.

**Sources of Test Compounds**

Camphor, 1,8-cineole, sesame oil, and some artemisinin were obtained from Sigma Chemical Co.7 Artemisinin was also obtained through the auspices of P. Olliaro.8 Essential oil from *A. annua* was generously provided by D. J. Charles.9

**Artemisia annua**

Five-week-old seedlings, furnished by John Teasdale,10 were planted the first week in July, 1994. Plants were harvested the first week in September, 1994 when flower buds had not yet opened, and were hung upside down in bunches to dry for 3 wk in a dark, ventilated building. Leaves were stripped from the stems, crumbled, sieved through a 4.75-mm mesh screen, and stored over a desiccant at 4 C until analyzed and used.

**Analysis of artemisinin in *A. annua***

Three samples from different parts (top, middle, and bottom) of the batch of harvested leaves were taken for analysis. Samples were dried, ground to a powder, and extracted with acetonitrile. Extracts were filtered, dried under vacuum, and stored at −10 C until analyzed following methods of Zhao and Zeng (1985) and Liersch et al. (1986). Basically, residues were dissolved in reagent alcohol, hydrolyzed with 0.2% NaOH, and the samples neutralized with 0.2 N acetic acid. The qinghaosu 260 (absorption maximum 262 nm) formed from each sample was analyzed in triplicate by HPLC on a 10 cm × 8 mm (I.D.) Nova-Pak C18 4 μm column with guard filter, using a Waters Associates11 system having a 990 photodiode array detector. The mobile phase was 10 mM potassium phosphate buffer (pH 7.9):methanol (67:33) at a flow rate of 1.5 mL/min and an injection volume of 25 μL. The system was calibrated by quantifying the qinghaosu 260 produced from base hydrolysis of pure artemisinin standard solutions of 0.009 and 0.018 mM. The average artemisinin content of the *A. annua* leaf preparation was determined to be 0.034% ± 0.001 SD.

**Experimental Protocols**

**Experiment 1.** This experiment was run to determine whether feed supplemented with dried leaves of *A. annua* would be active in reducing effects of infection with *E.
maxima or a mixed infection of E. tenella and E. acervulina. The following dietary levels of dried A. annua leaves were tested: 0, 0.5, 1, and 5% by weight. Chicks were randomly assigned to these diet treatments that were fed from 1 d of age through 3 wk of age. At 2 wk of age, chicks in each diet treatment were divided into groups of six chicks each. Chicks in one group in each diet treatment were each challenged with an oocyst suspension containing 5,000 oocysts E. maxima or a suspension of mixture of 50,000 oocysts E. tenella and 100,000 oocysts E. acervulina. One group of chicks from each diet treatment remained uninoculated controls. Weight gains and lesion scores were determined at 6 d PI.

Experiment 2. This experiment was run to determine whether starter diet supplemented with 1% dried A. annua would have an adjuvant effect in chicks immunized with the live vaccine IMMUCOX®, and raised on litter. Day-old chicks were placed on litter, and immunized by oral administration of IMMUCOX®. Chicks (16) in one pen were given regular starter feed. Chicks (18) in the other pen were given feed supplemented with 1% A. annua leaves. At 2 wk of age, half the chicks in each pen were removed to suspended cages (four or five chicks per cage) in order to remove them from the source of oocysts, but they continued to consume the original diet. The remainder of the chicks stayed on the litter through the end of the experiment (5 wk). An additional group consisting of eight chicks was placed in a brooder, given regular feed, and removed to suspended cages at 2 wk. At 4 wk of age, all chicks were challenged with a mixture of E. acervulina and E. tenella. Weight gains and lesion scores were determined at 6 d PI.

Experiment 3. This experiment was run to test some individual compounds present in A. annua leaves for anticoccidial activity against E. acervulina and E. tenella. Based on the analysis of artemisinin in the leaves of A. annua, it was determined that the amount of artemisinin in feed supplemented with 5% A. annua leaves was approximately 17 ppm. Based on reported analyses of other constituents (Charles et al., 1991), it was determined that 1,8-cineole and camphor could each be present at about 119 ppm in a 5% leaf supplement, and that the essential oils might approximate 264 ppm. Diets consisting of starter feed containing these amounts were mixed as described above, and fed to chicks from 1 d of age through 3 wk of age. At 2 wk of age, chicks were weighed, and one half the chicks within each diet treatment were inoculated by gavage with a 1-mL suspension containing 100,000 oocysts E. acervulina and 50,000 oocysts E. tenella. The other half of each diet treatment remained uninoculated controls. Weight gains and lesion scores were determined at 6 d PI.

Experiment 4. This experiment was run to more closely define the effective dose range of artemisinin and to determine possible differences in activity against single and mixed species infections with E. acervulina and E. tenella. Artemisinin was mixed with starter feed in the following concentrations: 0, 2, 8.5, and 17 ppm. Chicks were randomly assigned to and fed these diets for 4 wk. At 3 wk, chicks within each diet treatment were weighed and divided on the basis of weight into four infection groups: 1) uninfected controls, 2) infected with E. acervulina (100,000 oocysts per chick), 3) infected with E. acervulina (50,000 oocysts) plus E. tenella (20,000 oocysts), and 4) 50,000 oocysts E. tenella. Oocyst output per chick per group over the 16 h prior to necropsy was determined.

### Statistical Analyses

Data were examined by analysis of variance using the General Linear Models procedure of SAS® (SAS Institute, 1990). Significant differences among group means were determined using Duncan’s multiple range test.

### RESULTS

#### Experiment 1

Supplementation of broiler starter diet with up to 5% A. annua leaves did not significantly affect weight gain of uninfected chickens during the 6 d PI. All infected chickens had significantly reduced weight gains compared to their uninoculated dietary controls. Chickens that consumed the 1 and 5% supplements and that were

### TABLE 1. Effects of diets supplemented with Artemisia annua leaves on weight gains of coccidia-infected chickens at 6 d postinoculation, Experiment 1

<table>
<thead>
<tr>
<th>Percentage dietary A. annua leaves (%)</th>
<th>Uninoculated groups</th>
<th>Infected groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g) (n)</td>
<td>Eimeria maxima (g) (n)</td>
</tr>
<tr>
<td>0</td>
<td>112 ± 6a 6</td>
<td>82 ± 4b 6</td>
</tr>
<tr>
<td>0.5</td>
<td>103 ± 3a 6</td>
<td>79 ± 2c 6</td>
</tr>
<tr>
<td>1</td>
<td>111 ± 4a 6</td>
<td>66 ± 4b 6</td>
</tr>
<tr>
<td>5</td>
<td>106 ± 2a 6</td>
<td>69 ± 5c 6</td>
</tr>
</tbody>
</table>

a–dMeans ±SEM in a column with no common superscript differ significantly from the uninoculated control (P ≤ 0.05).
infected with *E. maxima* gained significantly less than chicks that had consumed the unsupplemented diet. Chicks that had consumed the 0.5 and 1% supplements and were infected with *E. acervulina* and *E. tenella* gained significantly less than those on the unsupplemented diet. However, chicks that had eaten the 5% supplement gained about the same as those on the unsupplemented diet (Table 1).

The mean lesion scores of the groups infected with *E. maxima* were uniformly low and were not significantly different from one another (Table 2). Lesion scores attributable to *E. acervulina* infection were significantly higher in chicks that had consumed 0.5 and 5% supplements than in chicks on the unsupplemented diets. Cecal lesion scores attributable to *E. tenella* infection were significantly lower in chicks that had eaten the 5% supplemented diet than in the infected groups on the other dietary treatments (Table 2).

### Experiment 2

Chickens immunized with IMMUCOX® that consumed an unsupplemented diet and that were removed from the litter at 2 wk gained the least weight and had the most severe cecal lesions upon challenge. However, statistically, weight gains and lesion scores were not significantly different from those of the unimmunized, unsupplemented controls (Table 3). On the other hand, immunized chicks removed from the litter at 2 wk that had consumed the 1% *A. annua* diet had the highest weight gain of all treatment groups, and significantly lower lesions than the parallel group on the unsupplemented diet (Table 3). Immunized chicks on the supplemented diet that had remained on the litter (5 wk) had numerically higher weight gains and lower cecal lesion scores than the similarly immunized group on the unsupplemented diet.

Over all, however, there were no statistical differences among the groups fed 1% *A. annua* and the group fed regular feed and kept on the litter 5 wk (Table 3).

## Experiment 3

Among the uninfected groups of chicks, there were no significant differences between the mean weight gain of the chicks on the unsupplemented diet and the mean gains of the chicks on any of the supplemented diets. However, those that consumed the artemisinin-supplemented diet gained the least, significantly less than chicks on the diets supplemented with camphor, 1,8-cineole or essential oil (Table 4). Infection with a mixture of *E. acervulina* and *E. tenella* caused significant reduction in mean weight gain in the group on unsupplemented feed and groups on feed supplemented with 1,8-cineole or essential oil (Table 4). The dietary supplement of camphor significantly reduced lesion scores attributable to *E. acervulina*. Diets supplemented with artemisinin, camphor, and 1,8-cineole significantly reduced lesion scores attributable to *E. tenella* (Table 4).

## Experiment 4

Supplementation of starter diet with artemisinin had no significant effect at any level on weight gain of the unchallenged chickens during the challenge infection. In general, challenge infection numerically lowered mean weight gains, but only chicks eating the unsupplemented diet that were inoculated with *E. tenella* alone had a mean weight gain significantly lower than its uninfected dietary control (Table 5).

Enumeration of oocyst output during the 16 h before the end of the experiment showed that artemisinin at all

### TABLE 3. Effect of supplementing feed with 1% *Artemisia annua* leaves on challenge infection of chickens immunized with the live vaccine IMMUCOX®, Experiment 2

| IMMUCOX® immunization (wk) | 1% supplement *A. annua* leaves | Chicks per treatment (n) | Weight gain (g) | Lesion score
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>–</td>
<td>8</td>
<td>81 ± 6ab</td>
<td>2.75 ± 0.16ab</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>8</td>
<td>78 ± 15b</td>
<td>2.75 ± 0.16ab</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>8</td>
<td>93 ± 15ab</td>
<td>1.22 ± 0.28ab</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>9</td>
<td>120 ± 15a</td>
<td>1.88 ± 0.30ab</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>9</td>
<td>104 ± 6ab</td>
<td>1.44 ± 0.18b</td>
</tr>
</tbody>
</table>

### TABLE 2. Effects of diets supplemented with *Artemisia annua* leaves on lesion scores of chickens infected with *Eimeria maxima* or *Eimeria acervulina* and *Eimeria tenella*, Experiment 1

Percentage dietary

<table>
<thead>
<tr>
<th>A. annua leaves (%)</th>
<th>E. maxima1</th>
<th>E. acervulina2</th>
<th>E. tenella3</th>
<th>(score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0 ± 0</td>
<td>2.20 ± 0.20ab</td>
<td>2.70 ± 0.33ab</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>1.0 ± 0</td>
<td>2.80 ± 0.20ab</td>
<td>2.40 ± 0.24a</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.0 ± 0.26</td>
<td>2.33 ± 0.21ab</td>
<td>2.33 ± 0.21a</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.0 ± 0.32</td>
<td>2.83 ± 0.41a</td>
<td>1.50 ± 0.22ab</td>
<td></td>
</tr>
</tbody>
</table>

a,b Means ± SEM within columns with no common superscript differ significantly (P ≤ 0.05).

1Site of lesions is the mid small intestine.
2Site of lesions is the upper small intestine.
3Site of lesions is the ceca.
supplement levels significantly reduced oocyst output of both *E. acervulina* and *E. tenella* (Table 6).

Regression analysis of oocyst output versus dietary level of artemisinin gave the following $R^2$ values for the different infections: *E. acervulina* alone, 0.9560 ($P \leq 0.001$); *E. acervulina* plus *E. tenella*, 0.5349 ($P \geq 0.001$); *E. tenella* alone, 0.5198 ($P \geq 0.001$), indicating linear relationships between artemisinin dose and oocyst output.

**DISCUSSION**

The initial hope underlying these experiments was to find a natural product with anticoccidial properties that could be used as a feed additive with minimal processing. Therefore, the first experiments were run to determine whether leaves of *A. annua* incorporated into regular feed would have anticoccidial activity. This plant was of great interest because it is known to contain artemisinin, a compound with antimalarial activity attributable in part to its potential to elicit oxidative stress (Krungkrai and Yuthavong, 1987; Levander et al., 1989; Meshnick et al., 1989), and because we have shown that oxidative stress (dietarily induced with n-3 fatty acids) is effective in controlling cecal coccidia (*E. tenella*) (Allen et al., 1996).

There is a global interest in growing *A. annua* as a commercial crop for both artemisinin and essential oil production (Ahmad and Misra, 1994; Laughlin, 1994; Woerdenbag et al., 1994; Fulzele et al., 1995). However, the artemisinin content of the leaves among different accessions has been found to vary widely. For example, one study of 18 accessions reported a range of artemisinin levels of 0.003 to 0.21% dry weight (Charles et al., 1990), whereas another study of 10 accessions reported a range of 0 to 0.1% dry weight (Liersch et al., 1986). The reported levels of artemisinin in leaves of accessions originating from the Washington, DC area were 0.04 to 0.11% dry weight (Liersch et al., 1986; Charles et al., 1990). Thus, the artemisinin level of the *A. annua* leaf tissue used in this study, 0.034% was similar to the lower levels previously reported for accessions from the Washington DC area (Charles et al., 1990). No pesticides were used on this crop.

Experiment 1 was basically a survey to test the effectiveness of different levels of *A. annua* supplementation on primary infections of three coccidia species that parasitize different segments of the chick small intestine: *E. acervulina*, the duodenum, *E. maxima*, the mid small intestine, and *E. tenella*, the ceca. Both the *E. maxima* infection and the dual *E. acervulina* and *E. tenella* infection were considered to be acute with respect to reduction in weight gain (Table 1). *Artemisia annua* appeared to have no protective effect against infection with *E. maxima*, as judged particularly by the significantly reduced weight gains seen in chicks that ate feed with the 1 and 5% supplements (Table 1). However, the low and unvarying lesion scores from these infections suggest the presence of an unidentified species in the *E. maxima* oocyst preparation (Table 2). In the dually infected chicks, diet supplementation with *A. annua* did not protect against weight gain reduction, which was drastic (ranging from about 50 to 70%) and

**TABLE 4. Effects of diets supplemented with individual constituents of *Artemisia annua* on weight gains and lesion scores 6 d postinoculation with *Eimeria acervulina* and *Eimeria tenella*, Experiment 3**

<table>
<thead>
<tr>
<th>Dietary supplement</th>
<th>Weight gains</th>
<th>Lesion scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected</td>
<td>Per treatment</td>
</tr>
<tr>
<td>(ppm)</td>
<td>(g)</td>
<td>(g)</td>
</tr>
<tr>
<td>0</td>
<td>90 ± 3abc</td>
<td>10</td>
</tr>
<tr>
<td>Artemisinin, 17</td>
<td>79 ± 8c</td>
<td>10</td>
</tr>
<tr>
<td>Camphor, 119</td>
<td>96 ± 3abc</td>
<td>10</td>
</tr>
<tr>
<td>1.8-Cineole, 119</td>
<td>98 ± 6d</td>
<td>10</td>
</tr>
<tr>
<td>Essential oil, 264</td>
<td>95 ± 4ab</td>
<td>10</td>
</tr>
</tbody>
</table>

*a-dMeans ± SEM within a parameter with no common superscript differ significantly ($P \leq 0.05$).

**TABLE 5. Effects of varying concentrations of dietary artemisinin on weight gains at 6 d postinoculation with coccidia, Experiment 4**

<table>
<thead>
<tr>
<th>Dietary artemisinin</th>
<th>Uninfected</th>
<th><em>Eimeria acervulina</em></th>
<th><em>Eimeria acervulina</em> and <em>Eimeria tenella</em></th>
<th><em>Eimeria tenella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>(ppm)</td>
<td>(g) (n)</td>
<td>(g) (n)</td>
<td>(g) (n)</td>
<td>(g) (n)</td>
</tr>
<tr>
<td>0</td>
<td>112 ± 2a</td>
<td>10</td>
<td>107 ± 4abcd</td>
<td>104 ± 4abcd</td>
</tr>
<tr>
<td>2</td>
<td>106 ± 2abcd</td>
<td>10</td>
<td>95 ± 2cd</td>
<td>93 ± 2a</td>
</tr>
<tr>
<td>8.5</td>
<td>109 ± 3abc</td>
<td>10</td>
<td>102 ± 5abcd</td>
<td>97 ± 4abcd</td>
</tr>
<tr>
<td>17</td>
<td>109 ± 4abc</td>
<td>10</td>
<td>111 ± 10abcd</td>
<td>98 ± 4abcd</td>
</tr>
</tbody>
</table>

*a-dMeans ± SEM with no common superscript differ significantly ($P \leq 0.05$).
The higher lesion scores attributed to *E. acervulina* in chicks fed the *A. annua*-supplemented diets (Table 2) suggested that *A. annua* was not protective against *E. acervulina* infection. On the other hand, lesion scores attributed to *E. tenella* were significantly reduced in chickens that had consumed the 5% supplemented diet (Table 2), suggesting that *A. annua* might afford some protection against *E. tenella*.

Experiment 2 was designed as a model to test effects of various treatments on partial and full protective immunity to coccidia provided by a live vaccine, IMMUCOX®. With live vaccination, protective immunity should develop over a period of 4 to 5 wk and it should occur gradually as a result of exposure of chicks to small but persistent levels of infection generated by recycling of the parasite from droppings in the litter. Removal of vaccinated chicks from the litter at 2 wk should allow only partial immunity to develop. The degree of developed immunity is tested by challenge infection at 4 wk with one or more of the coccidia species contained in the live vaccine. In this experiment, challenge infection was with a mixture of *E. acervulina* and *E. tenella*, in a dose calculated to provide moderate infection. Both the weight gain and lesion score data (Table 3) strongly suggest that dietary supplementation with 1% *A. annua* leaves provided significant protection against challenge infection, particularly for the partially immunized chicks. The lesion scores of the chicks immunized for 5 wk suggest that immunization was more effective against *E. acervulina* than *E. tenella* in chicks on regular feed. The low cecal lesion scores of fully immunized chicks on the 1% *A. annua* supplement are consistent with observations in Experiment 1.

The results from Experiments 1 and 2 suggest that *A. annua* leaves contain a component that offers protection against coccidiosis, that at a dietary level of 5% it can reduce cecal lesions during an acute primary infection with *E. tenella*, and at a lower level can enhance immunity towards both *E. acervulina* and *E. tenella* as developed by live oocyst vaccination.

One of the suppositions of this set of experiments was that artemisinin, the antimalarial component of *A. annua*, would also exhibit anticoccidial activity. However, the proper dose range to test in chickens was not known. From the analyzed content of the *A. annua* leaf harvest, it was calculated that the amount of artemisinin in a 5% supplemented diet was about 17 ppm. In Experiment 3, the protective effect of artemisinin was suggested by both weight gain and lesion score data (Table 4). Although unchallenged chicks that consumed the 17 ppm supplement over 3 wk gained the least during the infection period, challenge infection did not further reduce the weight gain. On the other hand challenged chicks on the control diet had a reduced weight gain of about 50%. The significant decrease in lesion scores attributable to *E. tenella* in chicks on the 17 ppm artemisinin diet is consistent with results from Experiments 1 and 2.

There are other components in the leaves of *A. annua* that are present in much greater quantities than artemisinin. These include the major components of its essential oil, artemisia ketone, camphor, and 1,8-cineole (Charles *et al.*, 1991). Camphor and 1,8-cineole are frequent components of aromatic medicinal herbs, and are reported to have antimicrobial properties (Hammerschmidt *et al.*, 1993; Carson and Riley, 1995). Because they were readily available commercially, they were also tested for anticoccidial activity. The levels used were rough estimates of possible amounts present in feed supplemented with 5% *A. annua* as calculated from published values (Charles *et al.*, 1991). Crude essential oil, as a source of artemisia ketone was also tested.

Both camphor and 1,8-cineole-supplemented feed helped maintain weight gain during challenge infection as compared to unsupplemented feed, whereas essential oil supplement did not (Table 4). The camphor-supplemented diet significantly protected against both *E. acervulina* and *E. tenella* lesions, whereas 1,8-cineole significantly protected against *E. tenella* lesions only. In fact, this compound exacerbated lesion scores attributable to *E. acervulina*. The essential oil supplement exacerbated lesions attributable to *E. tenella* (Table 4). These results suggest that some of the anticoccidial effects of *A. annua* leaves, particularly against *E. tenella*, could also be attributable to their content of camphor and 1,8-cineole.

In Experiment 4, pure artemisinin was tested at several levels against both single infections with *E. acervulina* and *E. tenella*, as well as a dual infection. These infections were judged to be mild because of the general lack of effect on weight gain (Table 5). Nevertheless, artemisinin-supplemented diets significantly reduced oocyst output in each infection in a dose dependent manner (Table 6). The effect on *E. acervulina* oocyst output was surprising, as lesion score data from the other experiments suggested that artemisinin was not effective against this parasite. The subjective nature of lesion scoring, including the fact that it incorporates some judgment of host inflammatory reaction, may partially explain the discrepancy. On the other hand, artemisinin was fed for a longer time in Experiment 4.

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**TABLE 6. Effects of varying dietary concentrations of artemisinin on oocyst output per chick at 6 d postinfection with coccidia**

<table>
<thead>
<tr>
<th>Dietary artemisinin (ppm)</th>
<th><em>Eimeria acervulina</em> (output × 10⁸)</th>
<th><em>Eimeria acervulina</em> and <em>Eimeria tenella</em> (output × 10⁸)</th>
<th><em>Eimeria tenella</em> (output × 10⁸)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30.6 ± 0.5a</td>
<td>17.6 ± 0.9a</td>
<td>2.7 ± 0.1a</td>
</tr>
<tr>
<td>2</td>
<td>27.1 ± 1.0b</td>
<td>12.7 ± 1.0b</td>
<td>0.95 ± 0.12b</td>
</tr>
<tr>
<td>8.5</td>
<td>20.7 ± 1.4f</td>
<td>13.0 ± 1.0b</td>
<td>0.46 ± 0.03f</td>
</tr>
<tr>
<td>17</td>
<td>6.2 ± 0.8d</td>
<td>9.9 ± 0.3c</td>
<td>0.45 ± 0.05c</td>
</tr>
</tbody>
</table>

a-dMeans ± SEM within a column with no common superscript differ significantly (*P* ≤ 0.05).

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than in Experiment 3. Perhaps this longer time is required for activity against \textit{E. acervulina}.

The level of artemisinin that was effective in lowering lesion scores and oocyst output in coccidia infections appeared to be much lower than that required to clear \textit{Plasmodium} infections in rodents and monkeys and humans. Rough calculations incorporating approximate daily feed consumption and body weight (both change during growth of chicks) indicate that chicks that consumed 17 ppm artemisinin in the feed actually ingested the compound at a rate 4 to 5 mg/kg body weight per d. At time of challenge at 2, 3, or 4 wk they would have ingested a total of about 70, 105, and 140 mg/kg, respectively.

As reviewed by Klayman (1985), a median effective oral dose of artemisinin at 138.8 mg/kg per d for 3 d cleared \textit{Plasmodium berghei} infections in mice; 200 mg/kg per d for 3 d cleared \textit{Plasmodium cynomolgi} in monkeys, which however, suffered relapse; treatment of human cerebral malaria (\textit{Plasmodium falciparum}) patients with a regimen of 600, 300, and 300 mg on three successive days (intragastrically by means of a nasal catheter) had a cure rate of about 90%. Interestingly, treatment with 200 mg/kg, respectively.

Whether the differences in oral dose levels required for anticoccidial and antimalarial activities reflect differences in modes of action against the two parasites, or differences in the metabolism of artemisinin in chickens and rodents is not known. In all these experiments artemisinin or leaves containing artemisinin were given prophylactically to prevent or reduce effects of a challenge coccidia infection rather than to effect a cure of an existing infection. Perhaps under these conditions lower daily levels of the compound would be needed.

Although dried leaf supplements of \textit{A. annua} were particularly effective in reducing lesion scores attributable to \textit{E. tenella} infections, the apparent lack of activity against other species as seen in Experiment 1 (possibly due to counteractive constituents) argues against its value as a feed additive. Further, the large variations in artemisinin and essential oil components, due to strain differences, climate, and soil (Charles et al., 1990, 1991), might make it very difficult to standardize. On the other hand, pure artemisinin shows some promise of broader activity against coccidiosis (Experiment 4) and deserves further study.

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REFERENCES


