Effects of Dietary Supplementation with n-3 Fatty Acid Ethyl Esters on Coccidiosis in Chickens

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ABSTRACT The ethyl esters of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids were added to a broiler starter diet singly or in combination [as bulk purified ethyl ester concentrate from menhaden oil (n3FAC)] in quantities similar to those found in a diet supplemented with 5% menhaden oil (MO). Diets were fed to chickens from 1 d of age through 3 wk of age. At 2 wk of age, the chickens were infected with Eimeria tenella, Eimeria acervulina, or Eimeria maxima. At 6 d postinfection (PI), the effects of the diets were assessed on weight gains, plasma carotenoids, gross lesion scores, and histological parasite scores in gut cross sections, or oocyst output. Significant ameliorating effects of diet on lesion scores and parasite scores were only seen in E. tenella infections and were only produced by the n3FAC and MO supplements. These two supplements, which contained higher molar concentrations of double bonds than the other supplements, also significantly reduced plasma carotenoids in uninfected chickens, indicating that they promoted a state of oxidative stress. These results are consistent with previous reports on the interaction of coccidiosis with dietary n-3 fatty acids and strengthen the hypotheses that dietary-induced oxidative stress is an effective deterrent against cecal coccidiosis in chickens.

(Key words: coccidiosis, n-3 fatty acids, eicosapentaenoic acid, docosahexaenoic acid, oxidative stress)

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INTRODUCTION

Previous investigations from this laboratory (Allen et al., 1996a, 1997a) have shown that chickens fed diets supplemented with sources rich in n-3 fatty acids (FA) had reduced gross lesion scores from acute Eimeria tenella infections but not Eimeria maxima infections. The tendency of these supplements to decrease plasma carotenoids in uninfected chickens (Allen et al., 1996b), suggested that oxidative stress, generated by peroxidation of the n-3 FA incorporated into chick tissues, played an important role in the effects of these diets. These results were consistent with those of Levander et al. (1992, 1993), who showed that diets containing high amounts of n-3 FA were protective for mice infected with Plasmodium yoelii.

As a further extension of our studies on avian coccidiosis, we have tested the anticoccidial activities of ethyl esters of the major n-3 FA found in menhaden oil (MO), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), individually, in combination, and in comparison with MO, in chickens infected with E. tenella, Eimeria acervulina, or E. maxima.

MATERIALS AND METHODS

Chickens and Housing

For Experiment 1, commercial male broiler chickens were obtained from a local producer. They were raised from 1 d of age in Petersime battery cages. For Experiment 2, male Sex Sals were obtained at 1 d of age, raised in Brower brooders through 2 wk of age, and then transferred to suspended wire cages. In both experiments, lighting was continuous, and room temperatures were maintained between 25 and 29 C.

Diets

Menhaden oil, bulk purified ethyl esters of EPA and DHA, and bulk purified n-3 FA ethyl ester concentrate (n3FAC) were obtained from the National Marine Fisher-

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Abbreviation Key: DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; FA = fatty acid; MO = menhaden oil; n3FAC = ethyl ester concentrate of menhaden oil; PI = postinfection.
The following laboratory strains of coccidia and oocyst doses per chick were used in Experiment 1: *E. acervulina* strain 12, $1 \times 10^5$ oocysts; *E. maxima* strain ES, $2.5 \times 10^4$ oocysts; *E. tenella* strain 80, $3 \times 10^4$. In Experiment 2, the dose per chick with *E. tenella* strain 80 was $4 \times 10^4$.

### Histological Parasite Scores

In Experiment 1, segments from infected areas of small intestine (from chickens infected with *E. acervulina* or *E. maxima*) or ceca (from chickens with *E. tenella* infections) were fixed in Carnoy’s fixative (Clark, 1981) embedded in paraffin, and 5 µ cross sections cut and stained with PAS\(^6\) (Clark, 1981). Ten cross sections per infected treatment group were microscopically examined and scored on a scale of 0 through 4 based on parasite density (percentage parasitized epithelium) per cross section: $1 = >0 \leq 25\%$, $2 = >25 \leq 50\%$, $3 = >50 \leq 75\%$, $4 = >75 \leq 100\%$.

### Oocyst Output

In Experiment 2, droppings were collected from each cage from 5 to 9 d PI. They were homogenized in water and diluted to 3-L vol. Quadruplicate counts on diluted homogenates were made using a McMasters chamber (Conway and McKenzie, 1991), and results reported in terms of oocyst output per chick.

### Plasma Carotenoids

Plasma carotenoids were determined according to Allen et al. (1996b).

### Statistical Analysis

Data were statistically analyzed using the General Linear Models program of SAS\(^7\) (SAS Institute, 1990), and significant differences among treatment group means determined using Duncan’s multiple range tests.

### Experimental Protocols

**Experiment 1.** Chickens were randomly grouped 10 per cage, and the groups assigned diet and infection treatments in a 5 (diet) by 4 (parasite) block design. Groups were placed on the designated diets from 1 d of age through 3 wk. At 2 wk of age, chickens were weighed and inoculated by gavage with sporulated oocysts. At 6 d PI, birds were weighed, bled, and killed, and the digestive tract scored for gross lesions (Johnson and Reid, 1970).

**Experiment 2.** Chickens were randomly assigned to diet treatments (control and 0.71% EPA) at 1 d of age, and they consumed these diets through 3 wk of age. At 2 wk of age, chickens within diet treatments were divided into two groups of eight per cage, based on weight (Gardiner and Wehr, 1950). Chickens in one group of each diet treatment were inoculated with $4 \times 10^5$ oocysts of *E. tenella* 80. At 6 d PI, chickens were weighed, bled, and killed, and the digestive tract scored for gross lesions.

### RESULTS

#### Experiment 1

Diet treatments had no significant effect on preinfection body weights (data not shown). During the 6-d infection period, *E. acervulina* and *E. maxima* infections significantly lowered weight gains, whereas *E. tenella* infection did not. Within a given parasite infection there was no influence of diet on weight gain (Table 2). However, diet and parasite infection significantly affected plasma carotenoid levels. The n3FAC- and MO-supplemented diets significantly lowered carotenoids in the uninfected controls and the *E. tenella*-infected chickens, whereas no significant diet effects were observed within the groups infected with *E. acervulina* and *E. maxima* (Table 2). Gross lesion scores in *E.
were no significant effects of diet on lesion scores of the
Dosage oocysts per chick 3
× 10^4
10
Control
247 ± 8 a±
229 ± 5 a±
219 ± 13 b±
186 ± 11 b±

Weight gain, g
10
Control
243 ± 12 a±
228 ± 11 a±
191 ± 8 a±
211 ± 11 b±

DHA
253 ± 6 a±
231 ± 6 b±
203 ± 11 b±
214 ± 20 b±

n3FAC
238 ± 9 b±
261 ± 31 a±
192 ± 12 b±
206 ± 14 b±

MO
250 ± 13 a±
242 ± 8 b±
213 ± 7 b±
230 ± 13 b±

Plasma carotenoids, µg/mL
10
Control
2.7 ± 0.5 t
2.5 ± 0.2 tu
0.8 ± 0.1 uyz
1.1 ± 0.1 yz

EPA
2.5 ± 0.2 tu
2.2 ± 0.3 tv
1.1 ± 0.1 k
0.7 ± 0.1 yz

DHA
2.4 ± 0.2 tv
2.1 ± 0.2 tv
0.8 ± 0.1 uyz
1.1 ± 0.2 k

n3FAC
1.8 ± 0.2 v
1.8 ± 0.2 v
0.5 ± 0.1 k
0.6 ± 0.1 yz

MO
1.9 ± 0.1 av
2.0 ± 0.2 av
0.4 ± 0.1 k
0.6 ± 0.1 yz

Gross lesion score
10
Control
NA^2
2.4 ± 0.3 x
3.1 ± 0.1 x
3.1 ± 0.2 x

EPA
NA
1.8 ± 0.3 xyz
2.9 ± 0.2 x
2.1 ± 0.3 x

DHA
NA
2.0 ± 0.3 y
2.8 ± 0.1 x
2.8 ± 0.4 x

n3FAC
NA
1.1 ± 0.4 x
3.2 ± 0.2 x
2.4 ± 0.3 x

MO
NA
0.9 ± 0.3 z
3.0 ± 0.2 x
2.5 ± 0.3 x

Parasite score
10
Control
NA
3.1 ± 0.5 x
2.4 ± 0.3 y
3.6 ± 0.3 y

EPA
NA
2.7 ± 0.6 x
2.4 ± 0.3 y
3.8 ± 0.1 x

DHA
NA
3.0 ± 0.5 x
3.2 ± 0.3 x
3.7 ± 0.2 x

n3FAC
NA
2.2 ± 0.5 x
2.3 ± 0.3 y
3.6 ± 0.2 y

MO
NA
2.0 ± 0.5 x
1.7 ± 0.2 y
2.8 ± 0.4 y

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Experiment 2

The diet supplemented with EPA had no significant effect on weight gain within either the control, or E. tenella-infected groups. However, the diet did reduce plasma carotenoids in the uninfected control chickens. Reductions in gross lesion scores and oocyst output were noted in the E. tenella-infected chickens (Table 3), but they were not statistically significant. A simultaneous light infection

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**TABLE 2. Effects of diets supplemented with n-3 fatty acid ethyl esters on several variables at 6 d postinfection with various coccidia**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chicks per treatment</th>
<th>Supplement</th>
<th>Uninfected</th>
<th>Eimeria tenella</th>
<th>Eimeria acervulina</th>
<th>Eimeria maxima</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage oocysts per chick</td>
<td></td>
<td></td>
<td>3 × 10^4</td>
<td>1 × 10^3</td>
<td>2.5 × 10^4</td>
<td></td>
</tr>
<tr>
<td>Weight gain, g</td>
<td>10</td>
<td>Control</td>
<td>247 ± 8 a±</td>
<td>229 ± 5 a±</td>
<td>191 ± 13 b±</td>
<td>186 ± 11 b±</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>EPA</td>
<td>243 ± 12 a±</td>
<td>228 ± 11 a±</td>
<td>191 ± 8 a±</td>
<td>211 ± 11 b±</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>DHA</td>
<td>253 ± 6 a±</td>
<td>231 ± 6 b±</td>
<td>203 ± 11 b±</td>
<td>214 ± 20 b±</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>n3FAC</td>
<td>238 ± 9 b±</td>
<td>261 ± 31 a±</td>
<td>192 ± 12 b±</td>
<td>206 ± 14 b±</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>MO</td>
<td>250 ± 13 a±</td>
<td>242 ± 8 b±</td>
<td>213 ± 7 b±</td>
<td>230 ± 13 b±</td>
</tr>
<tr>
<td>Plasma carotenoids, µg/mL</td>
<td>10</td>
<td>Control</td>
<td>2.7 ± 0.5</td>
<td>2.5 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>EPA</td>
<td>2.5 ± 0.2</td>
<td>2.2 ± 0.3</td>
<td>1.1 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>DHA</td>
<td>2.4 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>n3FAC</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>MO</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Gross lesion score</td>
<td>10</td>
<td>Control</td>
<td>NA^2</td>
<td>2.4 ± 0.3</td>
<td>3.1 ± 0.1</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>EPA</td>
<td>NA</td>
<td>1.8 ± 0.3</td>
<td>2.9 ± 0.2</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>DHA</td>
<td>NA</td>
<td>2.0 ± 0.3</td>
<td>2.8 ± 0.1</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>n3FAC</td>
<td>NA</td>
<td>1.1 ± 0.4</td>
<td>3.2 ± 0.2</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>MO</td>
<td>NA</td>
<td>0.9 ± 0.3</td>
<td>3.0 ± 0.2</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Parase score</td>
<td>10</td>
<td>Control</td>
<td>NA</td>
<td>3.1 ± 0.5</td>
<td>2.4 ± 0.3</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>EPA</td>
<td>NA</td>
<td>2.7 ± 0.6</td>
<td>2.4 ± 0.3</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>DHA</td>
<td>NA</td>
<td>3.0 ± 0.5</td>
<td>3.2 ± 0.3</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>n3FAC</td>
<td>NA</td>
<td>2.2 ± 0.5</td>
<td>2.3 ± 0.3</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>MO</td>
<td>NA</td>
<td>2.0 ± 0.5</td>
<td>1.7 ± 0.2</td>
<td>2.8 ± 0.4</td>
</tr>
</tbody>
</table>

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**TABLE 3. Effect of dietary supplementation of basal broiler starter diet with EPA ethyl ester on several variables measured at 6 d postinfection (PI) with Eimeria tenella**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chicks per treatment</th>
<th>Diet</th>
<th>Uninfected</th>
<th>Eimeria tenella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain, g</td>
<td>8</td>
<td>Control</td>
<td>76 ± 3 ab</td>
<td>71 ± 4 b</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>EPA</td>
<td>84 ± 3 a</td>
<td>72 ± 3 b</td>
</tr>
<tr>
<td>Carotenoids, µg/mL</td>
<td>8</td>
<td>Control</td>
<td>3.5 ± 0.3 a</td>
<td>2.9 ± 0.3 ab</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>EPA</td>
<td>2.5 ± 0.2 b</td>
<td>2.6 ± 0.2 b</td>
</tr>
<tr>
<td>Lesion score</td>
<td>8</td>
<td>Control</td>
<td>NA^3</td>
<td>3.2 ± 0.2 a</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>EPA</td>
<td>NA</td>
<td>2.6 ± 0.3 a</td>
</tr>
<tr>
<td>Oocyst output × 10^6, 5 to 9 d PI</td>
<td>8</td>
<td>Control</td>
<td>NA</td>
<td>170 ± 19.2 a</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>EPA</td>
<td>NA</td>
<td>136.7 ± 12.2 a</td>
</tr>
</tbody>
</table>

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^a,b^Means ± SEM within a variable with no common superscript differ significantly (P ≥ 0.05).
^c^Means ± SEM within variable columns with no common superscript differ significantly (P ≥ 0.05).
^d^Control = basal broiler starter ration; EPA = eicosapentaenoic acid ethyl ester supplement; DHA = docosahexaenoic acid ethyl ester supplement; n3FAC = n-3 fatty acid ethyl ester concentrate from menhaden oil supplement; MO = menhaden oil supplement.
^e^NA = not applicable.

tenella-infected chickens were significantly lowered by the diets supplemented with n3FAC and MO. Although no significant decreases in parasite scores were found, due to the large variation in count, a significant correlation was found between gross lesion scores and cross section microscopic parasite scores [r^2 = 0.99 (P ≥ 0.002)]. There were no significant effects of diet on lesion scores of the E. acervulina- or E. maxima-infected chickens (Table 2) and no correlations between lesion scores and parasite scores.
with *E. acervulina* (lesion scores of about 1) was observed in the *E. tenella*-infected chickens.

**DISCUSSION**

In a previous report, we found that diets supplemented with MO reduced lesion scores in *E. tenella*-infected chickens (Allen *et al*., 1996a). These diets also reduced plasma carotenoids in uninfected chickens (Allen *et al*., 1966b), a finding consistent with the hypothesis that the highly unsaturated n-3 FA contained in the MO can produce a state of oxidative stress detrimental to parasite development. The precedents for these experiments came from reports of Godfrey (1957, 1958) and Levander *et al.* (1992, 1993), which showed that diets supplemented with various fish oils were active in reducing infections of *Trichomonosoma* sp. and *Plasmodium* sp. That their antiparasitic effects could be reversed by additions of vitamin E also indicated that the effects were likely due to diet-induced oxidative stress.

As an extension of our earlier studies, the diets in Experiment 1 were formulated so that the EPA and DHA when added singly or in combination (n3FAC) provided the n-3 FA roughly equivalent to that found in a diet supplemented with 5% MO (Table 1), which had been found effective in reducing *E. tenella* gross lesion scores (Allen *et al*., 1996a). The results show that, singly, neither the EPA nor DHA was effective in reducing lesion scores of *E. tenella*-infected chickens, but together they had about the same effect as MO (Table 2). Thus, the anti-*E. tenella* activity observed roughly correlated with the degree of unsaturation (potential for oxidation) in the diet. The n3FAC and MO diets also significantly reduced plasma carotenoids in uninfected chickens (Table 2), again indicating that they were a source of oxidative stress. The results in Experiment 2 confirm that EPA by itself in a concentration similar to that in 5% MO was ineffective against *E. tenella* infections.

We have recently reported (Allen *et al*., 1997b) that the naturally occurring endoperoxide antimalarial, artemisinin, when used in low levels as a feed additive, also reduced *E. tenella* lesion scores. Its mode of action is also believed to be that of induced oxidative stress (Klayman, 1985; Krungkrai and Yuthavong, 1987; Levander *et al*., 1989; Meshnick *et al*., 1989). In general, then, it appears that the course of *E. tenella* infections, which proceeds in the relatively anaerobic environment of the cecum, can be tempered by diet-induced oxidative stress. On the other hand, this mode of action is not effective against *Eimeria* sp. such as *E. acervulina* or *E. maxima*, which carry out their life cycles in the upper mid-small intestine.

A number of studies have shown immunomodulating effects from feeding diets rich in n-3 FA centering around the capacity of the n-3 FA to reduce prostaglandin E (PGE) production through competition with arachidonic acid as a substrate for cyclooxygenase (Hwang, 1989; Lands, 1992; Zurier, 1993). In chronic inflammation, reduction of PGE by n-3 FA has an anti-inflammatory effect (Kelley *et al*., 1985). In infections, reduction of PGE by n-3 FA stimulates immunity by increasing tumor necrosis factor (Fritsche and Johnson, 1989; Wanatabe *et al*., 1993; Turek *et al*., 1994). Indomethacin is an inhibitor of cyclooxygenase, and thus will reduce PGE production (Wanatabe *et al*., 1993). In this respect, feeding n-3 FA and treating with indomethacin should have relatively similar effects on coccidia infections. In experiments to be published elsewhere (Allen, unpublished data), treatment of infected chicks with 5 mg/kg indomethacin did indeed reduce lesion scores and oocyst output from *E. tenella* similarly to MO and n3FAC in this present report. On the other hand, MO and indomethacin had different activity profiles against *E. acervulina* or *E. maxima* infections. Therefore, although oxidative stress appears to be a logical explanation for the actions of diets containing n-3 FA against *E. tenella*, an immunomodulating effect cannot be ruled out and needs to be studied in more detail (e.g., assaying for PGE and cytokine activity).

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**REFERENCES**


