Diets High in n-3 Fatty Acids Reduce Cecal Lesion Scores in Chickens Infected with *Eimeria tenella*

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**ABSTRACT**

Four experiments were run in which diets incorporating various levels of n-3 fatty acids (n-3FA) from fish (menhaden) oil or flax seed were tested for their abilities to reduce the severity of *Eimeria tenella* infections in young broiler chicks. The diets were fed from 1 d of age through 3 wk of age. Chickens were infected at 2 wk of age. Diets consisting of broiler starter ration supplemented with 2.5 to 10% fish oil, 10% flax seed oil, or 10% linseed oil significantly decreased cecal lesions and maintained weight gains as compared to unsupplemented diets. Light micrographs of ceca from chickens that consumed high n-3FA diets showed reduced parasite invasion and development. Short-term feeding of diets high in n-3FA to young chicks may be a practical method for reducing production losses from cecal coccidiosis.

(Key words: coccidiosis, oxidative stress, cecal lesion reduction, fish oil, flax seed)

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**INTRODUCTION**

Coccidiosis has been estimated to cost the American poultry industry about $300 million annually. About $90 million of this cost is for prophylactic medication with anticoccidial drugs. It has become increasingly clear, however, that many anticoccidials currently in use have diminished effectiveness because of the emergence of strains that are drug resistant. Consequently, new approaches to the control of coccidiosis are being sought. Although considerable efforts are being made to develop vaccines, products useful against all species of coccidia are not currently available.

Research on another protozoan parasite, *Plasmodium yoelii* (Levander *et al.*, 1993), has shown that infection in mice can be controlled through feeding diets containing high levels of n-3 fatty acids (n-3FA) under a condition such as vitamin E deficiency (Levander and Ager, 1993). Such dietary modulation increases the well-known susceptibility of malarial parasites to oxidative stress (Buffinton *et al.*, 1988; Clark *et al.*, 1989). The working hypothesis to account for this control is that the n-3FA become incorporated into the host red cell and parasite membranes, thereby destabilizing these structures in regard to oxidative damage.

Although *Plasmodium* and *Eimeria* parasites are both of the phylum Apicomplexa (Baker, 1982) and have some morphologically similar asexual stages, their life cycles and habitats in their respective vertebrate hosts are quite different. A fair amount of literature exists on the lipid metabolism and oxygen sensitivity of *Plasmodium* sp. (reviewed by Levander and Ager, 1993), whereas there is virtually no such information available on the intracellular forms of *Eimeria* sp. Nevertheless, growth and development within host cells by both parasites involves a continual turnover of their membranes, the composition of which should be qualitatively and quantitatively influenced by the dietary fatty acids consumed by the host. Therefore, a series of experiments was undertaken to determine whether feeding diets high in n-3FA to broiler chickens would protect them against infection with the cecal parasite, *E. tenella*. In these experiments, the highly unsaturated oils were added directly to ground broiler starter ration. A precedent for this procedure was the demonstration of partial control of *P. yoelii* infections in mice fed a high n-3FA oil, menhaden oil, mixed directly with ground laboratory chow (Levander *et al.*, 1992).

**MATERIALS AND METHODS**

**Animals and Housing**

The chickens used in these experiments were of a broiler strain and were obtained at 1 d of age from a commercial producer. They were housed in groups of 10 per cage in starter batteries (51 x 91 x 20 cm) under constant lighting at a starting temperature of 32.3 C.
Temperatures were gradually decreased to between 25 and 28 C over the following 2 wk. Treatment groups were assigned randomly to the various locations within the battery. Chickens were allowed to consume experimental diets and water ad libitum from 1 d of age through 3 wk of age.

**Experimental Diets**

The basal control diet (SS) consisted of a commercial starter ration\(^1\) (24% protein, 4% fiber, 4% fat, 3.12 kcal TME/g, and which contained a reported 23.1 IU vitamin E/kg), ground to a mash to facilitate mixing. The following oils and their percentage additions (wt/wt) to the basal diet were: purified menhaden oil (FO)\(^2\) 1, 2.5, 5, 10, and 20%; medium chain triglycerides (MCT)\(^3\) 5, 10, and 20%; Flaxseed oil (FLX)\(^4\) 10%, and linseed oil (LIN)\(^5\) 10%. The TME for each of these oils is about 8.0 kcal/g. Whole flaxseed (FS)\(^6\) was mixed with the basal diet at 15% by weight. The TME of flaxseed is reported to be 3.75 kcal/g (Lee et al., 1995). Before mixing, the FO and FLX supplements were stored frozen to prevent auto-oxidation. Flax seed was refrigerated at 4 C, and MCT, saturated oil that served as a caloric control (Levander et al., 1992), and SS were stored at room temperature. Diets were mixed just prior to the beginning of experiments, and were stored in closed containers at 4 C until presented to the chickens.

**Parasite**

The laboratory strain, 80, of *E. tenella* was developed from a field strain obtained from the Delmarva peninsula. It was propagated from a single oocyst infection, and was maintained by periodic passage through chickens. Just prior to each experimental infection, oocyst preparations were titrated with respect to lesion score in separate groups of age-related birds in order to determine infectivity. From this information, doses of oocysts were chosen that would produce lesion scores in the range from 2.5 to 3.0 (moderate to heavy) in birds consuming control diets.

**Basic Animal Protocols**

Chickens within a diet treatment were divided into groups (usually 10 chicks per group) of equal mean weight at 2 wk of age. Chickens in half of the groups on each diet treatment (I) were inoculated with *E. tenella* by oral gavage. Chickens in the other half of the groups remained uninoculated controls (U) and were given gavages of water. Chickens were weighed just before inoculation and again just before termination of experiments at 6 d postinoculation (PI), to determine weight gains during infection. Chickens were bled by cardiac puncture, then killed by cervical dislocation in order to obtain tissues for analyses and to look for cecal lesions. These lesions were scored in a blinded manner on a scale from 0 to 4 according to Johnson and Reid (1970).

**Microscopy**

Cecal tissues were fixed in Carnoy’s fixative (Clark, 1981), and sections from paraffin blocks stained with periodic acid Schiff (PAS) stain\(^7\) for examination by light microscopy.

**Experimental Protocols**

**Experiment 1.** This experiment was run as a pilot study to determine whether progression of infection by *E. tenella* could be affected by high dietary levels of n-3FA. The effects of two dietary levels of FO, 10 and 20%, were contrasted with effects of similar levels of MCT, a fully saturated oil that served as a caloric control (Levander et al., 1992), as well as the basal SS control diet. The inoculating dose for infected chickens was 58,000 sporulated oocysts per chick, and experimental groups (one replicate) contained five chickens each.

**Experiment 2.** This experiment contrasted the effects of n-3FA from an animal (5 and 10% FO) and plant (10% FLX, 10% LIN) source using 10% MCT and SS diets as controls. The inoculating dose for infected chicks was 160,000 sporulated oocysts per chick, and experimental groups (one replicate) contained 10 chickens each.

**Experiment 3.** This experiment contrasted effects of n-3FA in FO and whole flax seed. The amount of flax seed added (15% by weight) was calculated to provide the equivalent amount of n-3FA as there would be in the 5% FO diet. Chicks were each inoculated with 50,000 sporulated oocysts. Experimental diet groups (one replicate) contained 10 chickens each.

**Experiment 4.** This experiment was run to determine more closely the range of FO effective in reducing cecal lesions in *E. tenella* infections, and compared dietary levels of 1, 2.5, 5, and 10% FO using 10% MCT and SS as control diets. The inoculating dose was 50,000 sporulated oocysts per chick. Experimental diet groups (one replicate) contained 10 chickens each.

**Statistical Analysis**

Data were statistically analyzed using the General Linear Models Program of SAS\(^®\) (SAS Institute, 1979). Significant differences among treatment groups were determined using Duncan’s multiple range test.

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\(^{1}\)Southern States Cooperative, Upper Marlboro, MD 20772.

\(^{2}\)Stabilized with 0.02% Tetrabromo-hydroquinone. Fish Oils Testing Materials Program of the National Institutes of Health and National Oceanic and Atmospheric Administration, Bldg. 31, Room 4B63, National Institutes of Health, Bethesda, MD 20892.

\(^{3}\)Meglyol 810, Huls America, Piscataway, N J 08855.

\(^{4}\)Barlean’s Organic Oils, Ferndale, WA 98248.

\(^{5}\)Obtained at local hardware store.

\(^{6}\)Omega flax variety, generously provided by John F. Carter through the North Dakota Agricultural Experiment Station, North Dakota State University, Fargo, ND 58107.

\(^{7}\)Tissues processed and stained by American HistoLabs, Gaithersburg, MD 20879.
TABLE 1. Effects of dietary supplementation with fish oil on weight gains and lesion scores in broilers infected with *Eimeria tenella*

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Birds per group</th>
<th>Supplement level (%)</th>
<th>Parasite treatment</th>
<th>Weight gain (g)</th>
<th>Lesion score</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>5</td>
<td>0</td>
<td>Uninfected</td>
<td>246 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>FO</td>
<td>5</td>
<td>10</td>
<td>Uninfected</td>
<td>242 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCT</td>
<td>5</td>
<td>20</td>
<td>Uninfected</td>
<td>251 ± 18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>Infected</td>
<td>282 ± 20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>Infected</td>
<td>149 ± 18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>Infected</td>
<td>131 ± 23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.4 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCT</td>
<td>5</td>
<td>10</td>
<td>Uninfected</td>
<td>215 ± 22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>Infected</td>
<td>175 ± 20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.8 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>Infected</td>
<td>145 ± 13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>Infected</td>
<td>162 ± 10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.4 ± 0.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-d</sup>Values represent the means ± SEM of n birds in one replicate. Means within a column with no common superscript differ significantly (P < 0.05).

1<sup>SS</sup> = basal starter diet; FO = fish oil; MCT = medium chain triglycerides.

RESULTS

**Experiment 1**

No significant differences in mean weight gains were found between the U and I groups (Table 1). However, mean weight gain of both U and I chickens consuming the 20% FO and 20% MCT diets were significantly less than that of U or I chickens consuming the control diet or diets supplemented with 10% FO or 10% MCT. Most importantly, mean lesion scores of I chickens consuming the 10 and 20% FO diets were significantly lower than those of chicks eating the SS or MCT diets (Table 1). Examination of cecal cross-sections stained with hematoxylin and eosin revealed reduced parasite invasion and development in ceca from chicks on the 10% FO diet (data not shown).

**Experiment 2**

With the exception of chickens on the LIN diet, there were no significant differences in mean weight gains between the U and I groups within a diet treatment. However, mean weight gains of the 10% MCT and 10% FO chicks were about 10% lower than gains of the other groups (Table 2). As in the first experiment, I chickens consuming the FO, FLX, and LIN diets had significantly lower lesion scores (Table 2) than the SS and MCT I groups. Reduced parasite invasion and development was again seen in cecal cross-sections from the groups with lower lesion scores.

**Experiment 3**

There were no significant differences in weight gain between U and I groups fed the SS, MCT, or FO diets (Table 3). No U FS Group was run. The mean weight gain of the I FS Group was significantly lower than that of the U SS group but not the I SS group. Mean lesion scores of the I FO and FS groups were significantly lower than scores of the I SS and I MCT groups (Table 3).

**Experiment 4**

As in the previous experiments, infection with *E. tenella* had little effect on body weight gain (Table 4). The 2.5% FO diet provided the highest weight gain in U chicks. The I chickens eating the 2.5, 5, and 10% FO diets had significantly lower lesion scores than those consuming the SS and 1% FO diets. In this experiment, the I 10% MCT Group had a lower mean lesion score than the SS Group.

Examination of PAS-stained cross-sections of ceca from chickens fed the different diets revealed that the high n-3FA diets could reduce the degree of parasitization as well as retard parasite development. Figure 1 shows typical development of *E. tenella* in a cecum from a chicken fed the SS (control) diet. The forms present are female gametes, the wall-forming bodies of which stain magenta, and some oocysts. Figure 2 shows a similar section from a chicken fed a 10% MCT diet. Basically, the same forms and numbers are present as in control tissues. On the other hand, Figure 3 shows a section from a chicken fed 5% FO (Experiment 4). Primarily, a small number of small schizonts are seen, and development to the sexual stages has been retarded. Figure 4 shows a section of tissues from an infected chicken fed 15% FS. Virtually no penetration and development of the parasite has taken place.

DISCUSSION

A prime diagnostic characteristic of *E. tenella* infections is the presence of bright red blood in droppings. This blood comes from hemorrhages created in infected ceca by the eruption from the submucosa of large second-stage schizonts, which occurs between 4 and 6 d PI. Hemorrhaged blood is also coagulated within the cecal lumen, where it is formed, along with sloughed epithelium and oocysts, into cecal cores that can greatly distend the ceca. Lesion scores with a range from 0 to 4 quantify the severity of infection, and reflect the extent of bleeding, size of cecal cores, and gross damage to the mucosa (Johnson and Reid, 1970).
In all experiments, FO diets consistently reduced lesion scores. Supplemental FO levels as low as 2.5% had significant effects when compared with the SS diet (Table 4). Further, FO diets appeared to decrease the degree of mucosal colonization by and development of the parasite (Figure 3). The FLX and LIN diets in Experiment 2 also reduced mean lesion scores as compared to the SS diet. Additionally, in Experiment 3, in which FS was fed, lesion scores were markedly reduced, and only a very few parasites could be detected in the tissues (Table 4, Figure 4). These results thus provide strong evidence that dietary supplements high in n-3FA from both fish and plant sources can provide some protection to young broiler chicks against cecal coccidiosis.

It is well documented (Edwards and Marion, 1963; Hulan et al., 1989; Phetteplace and Watkins, 1989, 1990, 1992; Hargis et al., 1991; Olomu and Baracos, 1991; Watkins, 1991) that chick tissues readily incorporate highly unsaturated fatty acids from supplemented feed. Both menhaden and flax oils have high percentages of highly unsaturated fatty acids (29.5 and 66%, respectively) that are primarily n-3 FA (74 and 80%, respectively) (Exler and Weihrauch, 1986). However, whereas virtually all the n-3 FA in flax oil is linolenic acid (LNA), only 5% of the menhaden oil is LNA. The rest of the n-3FA in menhaden oil consists of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. There is some evidence that the latter two acids are more readily incorporated into biological tissues than LNA (Sanders and Younger, 1981; Sanders and Rosnahai, 1983; Nettleton, 1991). However, because of their higher number of double bonds, they are more subject to oxidation and have a greater potential to create more metabolic oxidative stress (Muggli, 1989). Plasma tocopherol concentrations as well as those of carotenoids (primarily lutein) were significantly reduced by 10 and 20% FO diets in these experiments (Morris et al., 1995; Allen et
FIGURE 1. Periodic acid-Schiff-stained section of cecum from a chicken infected with *Eimeria tenella* and fed basal starter ration, 6 d postinfection (1,200×). Bar equals 16.7 μm. Many macrogametes (small arrow) and oocysts (large arrow) are seen within epithelial cells.

FIGURE 2. Periodic acid-Schiff-stained section of cecum from a chicken infected with *Eimeria tenella* and fed a 10% medium chain triglyceride diet, 6 d postinfection (1,200×). Bar equals 16.7 μm. Many macrogametes (small arrow) are seen within epithelial cells.

FIGURE 3. Periodic acid-Schiff-stained section of cecum from a chicken infected with *Eimeria tenella* and fed a 5% menhaden oil diet, 6 d postinfection (1,200×). Bar equals 16.7 μm. Only occasional parasite forms (small arrow) are seen within epithelial cells. A goblet cell is marked by an asterisk.

FIGURE 4. Periodic acid Schiff-stained section of cecum from a chicken infected with *Eimeria tenella* and fed a 15% flaxseed diet, 6 d postinfection (1,200×). Bar equals 16.7 μm. Only occasional parasite forms (small arrow) are seen within the epithelial cells. A goblet cell is marked by an asterisk.

*ah.,* unpublished data), suggesting that these diets did create some oxidative stress.

If the original hypothesis were correct, that dietary n-3FA may have anticoccidial properties because they lead to oxidative destruction of the parasite, then decreased parasite development or colonization at normal sites of infection might be expected. This was observed at the light microscope level (Figures 1 to 4).

Increased levels of dietary n-3FA do generate increased requirements for dietary vitamin E (Dam, 1962; Muggli, 1989), as the latter is the body's primary antioxidant (Packer, 1991). In the current experiments, diets high in n-3FA reduced plasma levels of vitamin E, yet they also reduced lesions (Morris *et al.*, 1995; Allen *et al.*, unpublished data). These results seem paradoxical when considering experiments reported by Colnago *et al.*
humoral immune responses. It is now known that the coccidial infection. Additionally, a number of other Nockles, 1975; Tengerdy and Brown, 1977) have demonstrated immunity-enhancing effects of supplementary vitamin E. against Escherichia coli infections in chickens. Those experiments may have been addressing primarily vitamin E. to improve the overall defense of chickens against coccidiosis in commercial flocks. However, it is becoming increasingly clear that dietary fats can act as immune modulators, affecting both humoral and cellular responses (Gershwin et al., 1985; Johnston, 1985, 1988). In chickens, diets high in n-3FA have been reported to both stimulate (Fritsche et al., 1991) or to have no effect on antibody responses to sheep red blood cells (Phetteplace and Watkins, 1992), and to reduce proliferative responses to mitogens (Fritsche et al., 1991). Eimeria tenella infections that take place in the ceca are not always accompanied by significantly reduced weight gains (Long et al., 1980). We have found this to be particularly true in fast-growing broilers (unpublished observations). This characteristic contrasts with that of coccidial infections of the small intestine, during which the major nutrient absorbing areas of the gut are physically and functionally disrupted. In this present series of experiments, effects of infection on body weight gain were erratic. In fact, diet composition seemed to have the most influence on weight gain. In particular, U chickens did not gain well on the 20% MCT or 20% FO diets (Experiment 1). Chickens on these diets exhibited reduced feed consumption (data not shown), apparently because the diets were not very palatable. Consequently, lower supplementation levels were chosen for more detailed study.

In conclusion, these experiments have shown that diets high in n-3FA will, when fed from 1 d of age, reduce lesion scores in young broiler chickens infected with E. tenella. Levels of FO, FLX, and LIN up to 10% of the diet did not adversely affect body weight gain during the first 6 d following infection. The suppressive effects of these diets on E. tenella development are consistent with what might be expected from a state of oxidative stress. Short-term use of these diets for young chicks might be developed into a practical alternative for coccidiosis control in commercial flocks.

**ACKNOWLEDGMENTS**

The authors gratefully acknowledge the excellent technical assistance of Virginia Morris, Elizabeth Sanders, Dana Teasdale, and Barbara Leotta.

**REFERENCES**


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**TABLE 4. Effects of dietary supplementation with varying levels of fish oil on weight gains and lesion scores in broilers infected with Eimeria tenella**

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Birds per group</th>
<th>Supplement level</th>
<th>Parasite treatment</th>
<th>Weight gain</th>
<th>Lesion score</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>10</td>
<td>0</td>
<td>Uninfected</td>
<td>267 ± 8abc</td>
<td>0c</td>
</tr>
<tr>
<td>MCT</td>
<td>10</td>
<td>10</td>
<td>Infected</td>
<td>229 ± 13bc</td>
<td>3.2 ± 0.2a</td>
</tr>
<tr>
<td>FO</td>
<td>10</td>
<td>1</td>
<td>Uninfected</td>
<td>253 ± 10bc</td>
<td>0a</td>
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<tr>
<td></td>
<td>10</td>
<td>2.5</td>
<td>Infected</td>
<td>258 ± 17bc</td>
<td>2.4 ± 0.27bc</td>
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<td>5</td>
<td>Uninfected</td>
<td>302 ± 9ab</td>
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<td>316 ± 8a</td>
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<td>242 ± 11c</td>
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<td>272 ± 18abc</td>
<td>1.56 ± 0.41d</td>
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a-dValues represent the means ± SEM of n birds in one replicate. Means within a column with no common superscript differ significantly (P < 0.05).

SS = basal starter ration; MCT = medium chain tryglycerides; FO = fish oil; FS = whole flaxseed.


