Research Paper

EFFECTS OF SUPPLEMENTATION OF BROILER DIETS WITH FISH OIL AND LINSEED OIL ON GROWTH PERFORMANCE, CYTOKINES, AND CECAL HISTOPATHOLOGICAL CHANGES IN BROILER CHICKENS INFECTED BY EIMERIA TENELLA

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Avian coccidiosis is one of the serious infectious diseases affecting poultry, caused by different species of genus Eimeria. The present study was designed to evaluate the impacts of supplementation of broiler diet with Fish Oil (FO) or Linseed Oil (LO) at 3 and 5% on growth performance, hematological parameters, cytokines, antioxidant capacity, fecal oocysts count, and cecal histopathological changes of broiler chickens infected by Eimeria tenella. One hundred eighty 1-day old Cobb chicks were randomly allocated to 6 groups (30 chicks each). Each treatment was replicated 3 times with ten chicks (n-10). The experimental (isocaloric- isonitrogenous) diets were based on corn-soybean meal with 3% corn oil (positive and negative control), FO at 3 and 5% and LO at 3 and 5%. Chickens were inoculated by gavage with 40,000 sporulated oocysts at 21 days of age. Feeding diets supplemented with 3 and 5% LO resulted in higher body weight and body weight gain than those fed FO supplemented diets or positive control group. Diets supplemented with 5% FO or 5% LO significantly (p< 0.05) reduced cecal lesions and parasitic density scores caused by E. tenella. Dietary supplementation of 3% LO significantly (P<0.05) decreased the fecal coccidial oocysts count through 12 days post coccidial infection followed by 5% LO supplementation compared with those of the broilers fed the negative control diet and other treatment groups. Hematological parameters, Red Blood Cell count (RBCs), Packed Cell Volume (PCV), blood hemoglobin (Hb) were significantly decreased in the positive control. However, there were no significant differences in hematological parameters between negative control and other experimental groups. Diets supplemented with 5% FO increased plasma level of interleukine-6 (IL-6) compared with other treatment groups, while 5% LO increased plasma level of IL-1 and tumour necrosis factor. Serum total antioxidant capacity in chickens fed diets supplemented with LO (3% and 5%) were higher than other groups. In conclusion, supplementation of broiler diets with 3-5% LO improved growth performance and reduced cecal lesions and fecal oocysts count and effective in controlling the adverse effects of coccidiosis with E. tenella.

Keywords: Broiler, Oils, Performance, Coccidiosis, Interleukines, Antioxidant

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INTRODUCTION
Avian coccidiosis is an intestinal disease caused by protozoan parasites of the genus *Eimeria* occurs worldwide. It is considered to be one of the most economically important diseases of domestic poultry. It costs chickens producers worldwide at least 3 billion $US annually (Dalloul and Lillehoj, 2005). There are nine different species of *Eimeria* are specific to chickens, including *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria maxima*, *Eimeria mitis*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mivati*, *Eimeria hagani*, and *Eimeria tenella* (McDougald and Fitz-Coy, 2008). Coccidiosis is highly host specific and found in the gut, where it develops and multiplies intracellularly. Parasites are transmitted among broilers by a fecal to oral route through the ingestion of sporulated oocysts (Dalloul and Lillehoj, 2005). The nine species of *Eimeria* infecting chickens are not equally important. *E. acervulina*, *E. maxima* and *E. tenella* are the most pathogenic species of *Eimeria*. *E. tenella* infections are found only in the caeca and it is called caecal coccidiosis (Mathis, 2005). Moreover, McDougald and Fitz-Coy (2008) postulated that *Eimeria tenella* causes a severe disease characterized by bleeding, hemorrhagic lesion development, high morbidity, mortality, and reduced weight gain. The study of the relationship between diet composition and coccidia is not new area of interest, but due to the development of the efficient, low-cost anticoccidial drugs caused lesser interest in dietary modulation. However, with the appearance of resistance to coccidiostats, the possible role of nutrition has recently attracted interest (Gabriel *et al*., 2006).

The n-3 fatty acids are polyunsaturated fatty acids, the major fatty acids being eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found abundantly in fish oil, and alpha-linolenic acid (ALA), being a major component of flaxseed oil. Several investigations (Allen *et al*., 1994, 1996a, 1997 and 1998; and Allen and Danforth, 1998) have shown that feeding diets rich with n-3 fatty acids from menhaden oil, expressed flaxseed oil and flaxseed significantly reduced cecal lesions caused by *Eimeria tenella* in broiler chickens from 1 day to 21 days of age. Stulnig (2003) reported that omega-3 fatty acids possess anti-inflammatory or less inflammatory properties by decreasing the release of pro-inflammatory eicosanoids and cytokines. Cytokines produced by white blood cells serve as regulators to the whole body by exertion of different effects on lymphocytes and other immune cells in response to infection and injury. From the human health aspect, omega-3 fatty acids are essential for playing important role in the prevention of coronary heart disease, hypertension, inflammatory, autoimmune disorders and cancer (El-Yamany *et al*., 2008).

Therefore, the objectives of the current study were to evaluate the effects of feeding diets supplemented with fish oil, linseed oil in comparison with corn oil on the growth performance and reduction of coccidiosis in broiler chicks.

MATERIALS AND METHODS
Experimental Birds and Dietary Treatments
One hundred eighty 1-day-old Cobb chicks were used. The chicks were reared under constant lighting and at a starting temperature 35-32 °C. Temperature was gradually decreased to between 25 and 28 °C over the following 2 wk. All the broiler chicks were fed a starter control diet from 1-7 days of age. At 8th day of age, the chicks were weighed and randomly allocated into six groups.
of equal body weights, each of 30 chicks, in 3 replicates each of 10 chicks per cage (150 x 100 cm) and were fed experimental starter diets from 8-14 days of age, experimental grower diets were fed from 14-28 days of age, then experimental finisher diets were fed from 28-33 days of age Table 1. The experimental isonitrogenous isocaloric corn-soybean meal based diets were supplemented with 3% corn oil (positive and negative control groups), fish oil and linseed oil at 3 and 5% for the coccidia infected groups. Feed and water were provided ad libitum. The chicks were not vaccinated against diseases. At day 21 (just before inoculation with *E. tenella*) and day 33 of the experiment, all of the experimental broilers were weighed and feed intake was measured. Body weight gain and feed conversion ratio (g feed/g gain) were calculated.

**Eimeria Infection and Assessment of Fecal Oocysts**

Before inoculation of the chickens with *Sporulated oocysts* of *Eimeria tenella* necropsy was run on three chicks per each group to make sure that the birds are free from any kind of coccidiosis. The chickens of each replicate of the dietary treatment groups were infected at the 21st day of age by oral inoculation with 40,000 sporulated oocysts of *E. tenella* per ml (Donal, 1989), 1 ml/chicken using graduate adjustable insulin syringe introduced directly into the crop

| Table 1: Ingredients (%) and Proximate Composition of the Experimental Diets |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Oil (%) Ingredients         | Experimental Diets* |                  |                  |                  |
|                             | 3     | 5     | 3     | 5     | 3     | 5     |
| Yellow corn (8.5%)          | 56.2  | 51.6  | 60.1  | 55.8  | 66.2  | 61.7  |
| Soybean meal (44%)          | 31.5  | 39.1  | 27.6  | 35.4  | 22   | 30   |
| Corn gluten (62%)           | 6     | 1     | 5.5   | 0     | 5.5   | 0     |
| Corn oil                    | 3     | 5     | 3     | 5     | 3     | 5     |
| Limestone                   | 2.2   | 2.2   | 1.5   | 1.5   | 1.5   | 1.5   |
| Dicalcium phosphate         | 0.5   | 0.5   | 1.7   | 1.7   | 1.3   | 1.2   |
| Vit. & Min. Premix          | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  |
| Salt                        | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   | 0.3   |
| DL-Methionine               | 0.15  | 0.15  | 0.12  | 0.12  | 0.12  | 0.14  |
| DL-Lysine                   | 0.1   | 0.1   | 0.1   | 0.1   | 0.1   | 0.05  |
| CP %                        | 22.99 | 23.1  | 21.17 | 21.06 | 19.00 | 18.98 |
| ME, Kcal/kg                 | 3092  | 3099  | 3129  | 3133  | 3197  | 2198  |
| Ca %                        | 1.01  | 1.05  | 1.02  | 1.04  | 0.9   | 0.91  |
| Available P %               | 0.47  | 0.48  | 0.45  | 0.46  | 0.35  | 0.36  |

Note: * positive and negative control diets were supplemented with corn oil at 3%, other treatment groups were supplemented with fish oil and linseed oil at 3 and 5%. † vitamins and minerals premixed to cover the required vitamins and minerals per each kilogram diet (Vit. A, 10000 I.U.; Vit. D3, 1500 I.U.; Vit. E, 10 mg; Vit. K3, 2 mg; Vit. B1, 2 mg; Vit. B2, 5 mg; Vit. B6, 3 mg; Vit. B12, 0.01 mg; Niacin, 27 mg; Follic acid, 1 mg; Biotin, 0.05 mg; Pantothenic acid, 10 mg; Mn, 60 mg; Zn, 50 mg; Cu, 10 mg; I, 0.1 mg; Se, 0.1 mg; Co, 0.1 mg; Fe, 50 mg).
of the chick. Samples of fecal material were collected from each cage tray (pool of three samples per tray in each replicate cage) at 6, 7, 8, 9, 10, 11, 12th day postinfection and the number of oocysts were assessed using a McMaster-chamber method (Conway and Mckenzie, 1991). The number of oocysts excreted was expressed as number of oocysts per gram of original sample and calculated according to the following formula:

No. of oocysts/gram faeces = No. of oocysts in 2 chambers x 50

**Fatty Acids Profile of Used Oils**

Samples of 250 microliter from well mixed fresh samples of each of corn oil, linseed oil, and fish oil (n = 6) were resolubilized into 2 ml of boron trifluoride-methanol-hexane solution (35% boron trifluoride, 45% methanol, 20% hexane). The tubes containing the resolubilized oil samples were heated in a water bath (90-100 °C) for 60 min. After cooling, 2 ml of hexane and 2 ml distilled water were added. The samples were mixed and allowed to separate. The hexane (upper) layer was withdrawn. Two µl of hexane layer was taken for separation of fatty acids by gas chromatography. Fatty acid analysis was performed with focus gas chromatograph (Thermo-nicolet, USA) equipped with TR-5 fused silica column (30 mm x 0.25 mm i.d.). The initial oven temperature was set at 110 °C, held for 0.50 min and then increased by 20 °C/min to 190 °C, and held for 7 min, then increased by 5 °C/min to 210 °C and held for 8 min and increased by 20 °C/min to 230 °C and held for 0.2 min (Cherian and Sim, 1992). Inlet and detector temperatures are 250 °C. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. Fatty acid methyl esters were identified by DSQ11 mass spectrometer.

**Hematological Assessement**

Blood samples were collected at 28th d of broilers age (7 days postinoculation of oocysts) from the brachial vein of 6 chickens per each experimental group using a 3 mL sterile syringe and a 23-gauge needle containing the anticoagulant, ethylene diamine tetraacetic acid. Blood hemoglobin (Hb) was assessed by Drabkin (Drabkin, 1964). Red Blood Cells count (RBCs), Packed Cell Volume (PCV), blood indices; Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined according to Jain (1986).

**Interleukines measurement**

Blood samples were collected at 28th d of the broiler age (7 days of oocyst infection) from the brachial vein of 6 chickens per each experimental group using a 3 mL sterile syringes with and without a 23-gauge needle containing the anticoagulant, ethylene diamine tetraacetic acid. Plasma and serum samples were centrifuged for 15 minutes at 1000 × g at 2-8 °C. Supernatant was stored at -20 °C until analysis. Interlukin (IL)-1, IL-6 and tumour necrosis factor α (TNF α) concentration were measured by IL-1, IL-6 and TNFα kits (GENORISE SCIENTIFIC, INC) according to methods described by Guida et al. (1992), Hong et al. (2007) and Brennan and McInnes (2008), respectively.

**Total Antioxidant Capacity (TAC)**

The serum levels of Total Antioxidant Capacity (TAC) were assayed by spectrophotometer using commercial kits (supplied by Biodiagnostic, Egypt) according to the methods adopted by Koracevic et al. (2001).

**Lipid Peroxidation (Malondialdehyde, MDA)**

Lipid peroxidation markers in serum were
determined using the thiobarbituric acid (TBA) test for malondialdehyde (MDA), using commercial kits (supplied by Bio-diagnostic, Egypt) according to the method of Stocks and Dormandy (1971).

**Histopathological Examination**

At 28 days of age, three chickens from each replicate of each experimental group were weighed, bled, killed, and the ceci were scored for gross lesions from zero to 4 according to Johnson and Reid (1970). Ceci were removed from the killed broilers (3 birds per cage, 9/group), rinsed with saline, and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 mm, and stained with hematoxylin and eosin (Bancroft et al., 1990). Six cecal sections from each chicken sample were mounted on a single slide and assigned as a score for the histopathological lesions and scoring the parasite lesions score. The mean score was then determined for each slide. The score for the histopathological lesions was assessed according to Korver et al. (1997) as follows: 0 = no lesions; 1 = mild inflammatory cell infiltrate in the mucosa with intact epithelium and no submucosal or muscularis involvement; 2 = extensive mucosal inflammatory cell infiltration and submucosal edema; 3 = as described above in (2) with inflammatory cell infiltration extending into the muscularis; and 4 = destruction of mucosa with necrosis and hemorrhage. Another score was given microscopically for the parasite density (percentage parasitized epithelium) per cross section on a scale of 0 through 4: 1 = >0 ≤ 25%, 2 = >25 ≤ 50%, 3 = >50 ≤ 75%, 4 = >75 ≤ 100% according to Allen and Danforth (1998).

**Statistical Analysis**

The results were subjected to a one-way ANOVA to test the impact of fish oil and linseed oil supplementation compared with corn oil supplementation (control) on growth performance, oocysts count, and cecal histopathological changes of broilers infected by E.tenella. Data were analyzed using statistical SPSS v20 (SPSS Inc., Chicago, IL, USA). Differences between dietary groups means were compared using Duncan’s multiple range test. Differences due to dietary treatments were considered significant if P-value for the effect was < 0.05.

**RESULTS AND DISCUSSION**

The non-infected group (negative control) was healthy and showed normal dropping, good appetite and good feathering. The infected group (Positive control) was severely affected and showed the most severe symptoms (depression, ruffled feather, anorexia and bloody diarrhea). The onset of clinical signs was in the 4th day postinfection and these signs were mild and deceased in the groups supplemented with Fish Oil (FO) or Linseed Oil (LO) at 3 and 5%.

Fatty acids composition percentage of the supplemented oils which were used in the experimental diets are presented in Table 2 revealed that corn oil is rich in linoleic acid (18:2n6) and low in α linolenic acid (18:3n3) which constituted 61.80 and 0.88%, respectively. In contrast the linseed oil found to be very rich in α linolenic acid content and is markedly high (64.12%). However, long chain fatty acids (EPA and DHA) are higher in fish oil than in the other experimental oils and constituted 10.65 and 8.64%, respectively.

**Growth Performance**

Omega 3 (ω-3) are polyunsaturated and essential fatty acids found abundantly in fish oil, flaxseed oil and whole flaxseed and can be easily
Table 2: Fatty Acid Composition Percentages of Experimental Oils Used in Broiler Diets

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>(%) of FA in Supplemented Oils</th>
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<tbody>
<tr>
<td></td>
<td>Corn Oil</td>
</tr>
<tr>
<td>Myristic (14:0)</td>
<td>0.19</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>12.13</td>
</tr>
<tr>
<td>Palmitoleic (16:1)</td>
<td>0.04</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>3.41</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>21.54</td>
</tr>
<tr>
<td>Linoleic (18:2 n-6)</td>
<td>61.8</td>
</tr>
<tr>
<td>&amp;-Linolenic (18:3 n-3)</td>
<td>0.88</td>
</tr>
<tr>
<td>Eicosonoic acid (20:1)</td>
<td>ND</td>
</tr>
<tr>
<td>Archidonic acid (20:4n6)</td>
<td>ND</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (EPA, 20:5n3)</td>
<td>ND</td>
</tr>
<tr>
<td>Docohepexanoic acid (DHA, 22:6n3)</td>
<td>ND</td>
</tr>
<tr>
<td>Σ SFA 1</td>
<td>15.73</td>
</tr>
<tr>
<td>Σ MUFA 2</td>
<td>21.58</td>
</tr>
<tr>
<td>Σ Omega 6</td>
<td>61.8</td>
</tr>
<tr>
<td>Σ Omega 3</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Note: ND = Not Detected; LO = Linseed Oil; FO = Fish Oil.
1SFA: Saturated Fatty Acids; 2MUFA: Monounsaturated Fatty Acids.

Supplementation of the basal diets with 3 and 5% LO significantly improved (P<0.05) Body Weight (BW) and Body Weight Gain (BWG) along the feeding trial (8-21 d) pre-infection and at (21 to 33 d) post-infection with *E. tenella*. However, FO supplementation (at 3 and 5%) did not improve BW and BWG more than the control groups fed corn oil (3%) supplemented diets. Infection of the broiler chickens with *E. tenella* decreased BW and BWG in the control diet and those fed the FO (at 3 and 5%) supplemented diets. While feeding the diets supplemented with FO maintained the higher BWG in spite of the infection with *E. tenella* at 21 d of age Table 3. Feed Conversion Rations (FCR) were lower in the broiler chickens fed the LO supplemented diets. Generally, infection with *E. tenella* (40,000 oocyst/bird) at 21 d of age decreased BW and BWG and increased the FCR in the broilers fed the control diet. These results are consistent with the finding of Lopez-Ferrer et al. (2001b) who observed higher weight gain in chickens fed diets high in LO levels. On the other hand, Murakami et al. (2010) found that there was a lower weight gain when broilers were fed diet supplemented with 6.5% linseed oil in the periods of 1-21 days of age. During the period 21-33 days of age (postcoccidial infection) there was a significant difference in the weight and body weight gain between positive and negative control groups indicating that *E. tenella* infection decreased body weight and body weight gain. However body weight development was improved in the broiler chickens fed diets supplemented with 3 and 5% LO. No significant differences in mean body weight and body weight gain were found between the broiler chickens fed diets supplemented with FO at 3 and 5% and the positive group (broilers fed diet supplemented with 3% corn oil and infected with *E. tenella*). Allen et al. (1996) found that the infection by *E. tenella* did not significantly reduce body weight. However, negative effects on broilers growth and performance upon including fish oil in broiler diets were reported (Saleh et al., 2009; and Aziza et al., 2014) and are in agreement with our current results. Moreover, *E. tenella* infection that take place in the ceca are not always accompanied by significant reduction in weight gains (Long
et al., 1980). This characteristic contrasted with that of coccidiosis infections of the small intestine, during which the major nutrients absorbing areas of the gut are physically and functionally disrupted (Allen et al., 1996). However, our results indicates that LO supplementation of broiler chicken diets reduced the severity of *E.tenella* infection and maintained proper growth.

**Oocysts Shedding**

Parasites are transmitted among broilers by a fecal to oral route through the ingestion of sporulated oocysts (Dalloul and Lillehoj, 2005). Moreover, Chapman et al. (2002) reported that there are many factors affecting the infectivity of sporulated oocysts, including number of oocysts present in litter, chick density, susceptibility of birds, and immunogenicity. Oocysts shedding has been shown to be a useful way to determine the level of *E.tenella* infection (Lee et al., 2009). The effect of feeding diets supplemented with FO or LO at 3 and 5% on oocyst numbers in the fecal material collected at d 6, 7, 8, 9, 10, 11, and 12th post-infection with *E. Tenella* is presented in Table 4. No oocysts were detected in fecal material obtained from the cages of the broiler chickens in the negative control group (non-infected group). Significant oocysts counts among different treatment groups starting from d 6th post infection through d 12th post infection (p< 0.05) are reported. The highest fecal oocysts count was found in the positive control group (infected chickens fed diet supplemented with CO at 3%), whereas the lowest count was observed in chickens fed the diet supplemented with LO at 3% followed by those fed 5% LO and 3% FO throughout the oocyst counting period. Oocysts shedding peaked between d 7th and 8th postinoculation Table 4. The allover effect of supplementing broiler diets with corn oil, FO and LO indicates the LO supplementation markedly decreased fecal shedding of *E.tenella* oocyst. Molan (2014) postulated that although the number

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary Treatments</th>
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<tr>
<td></td>
<td>Basal Control</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Preoccidial infection (8 to 21)</td>
<td></td>
</tr>
<tr>
<td>Body Wt (g) at 8 d</td>
<td>134</td>
</tr>
<tr>
<td>Body Wt (g) at 21 d</td>
<td>650±9.37b</td>
</tr>
<tr>
<td>Body Wt gain (g)</td>
<td>516±9.37b</td>
</tr>
<tr>
<td>FCR</td>
<td>1.43±0.04a</td>
</tr>
<tr>
<td>Postoccidial infection (21-33)</td>
<td></td>
</tr>
<tr>
<td>Body Wt (g) at 33 d</td>
<td>1320±28.8b</td>
</tr>
<tr>
<td>Body Wt gain (g)</td>
<td>670±16.5a</td>
</tr>
<tr>
<td>FCR</td>
<td>1.81±0.05b</td>
</tr>
</tbody>
</table>

Note: *control (negative and positive), FO3%, LO3%, FO5%, and LO5% represent corn-soybean meal basal diets containing 3% corn oil (negative and positive control), 3 and 5% fish oil, and 3 and 5% linseed oil. FO = Fish Oil; LO = Linseed Oil; Means ± SE per nearest gram.

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of oocysts shed by the infected birds is an important parameter, the most important factor in the epidemiology of coccidia is the ability of the oocysts to sporulate, which constitutes the reservoir of infection. In fact, coccidian infection occurs only when chickens ingest sporulated oocysts and infected birds excrete oocysts with their feces, of which a number will sporulate. Subsequently, sporulated oocysts are ingested by the same or other chickens, hereby spreading the infection. Consequently, the significant reduction in sporulation of the fecal oocyst shed by broilers fed diet supplemented with 3 or 5% LO as well as supplementation of the diet with 3% FO will lead to less contamination with infective coccidian oocysts.

Hematological Parameters
Hematological parameters were measured at 7 days post infection and are illustrated in Figure 1. There were significant decreases of RBCs count, Hb concentration, and PCV and Main Corpuscular Hemoglobin Concentration (MCHC) with an increase in the Main Corpuscular Volume (MCV) of chickens fed diet supplemented with 3% CO and infested with *E. tenella* (positive control). However, there were no significant differences in hematological parameters between negative control (uninfected group) and other experimental groups infected with *E. tenella* fed diets and supplemented with linseed oil or fish oil. The findings showed that feeding diets supplemented with LO and FO achieved and maintained blood parameters similar to that of the negative non infected control group. Iriazaary-Rovira (2004) and Wakenell (2010) showed that coccidiosis caused by *E. tenella* induced a higher reduction in TRBC (Total Red Blood Count) and PCV. These results are similar with those obtained by Fukata *et al.* (1997) who reported lower counts of TRBC and PCV in chickens infected with *E. tenella* when they compared to the uninfected controls. Also, Ogbe *et al.* (2010) reported a slight drop in the PCV, Hb and RBC counts in *E. tenella* infected broilers. Moreover, Razzaq *et al.* (2003) demonstrated low Hb and Total Erythrocyte Count (TEC) in quail chicks experimentally infected by *E. tenella*. Also, Yang *et al.* (2006) found that PCV value was higher in chickens fed diets supplemented with fish oil and

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corn oil. In the present study, supplementation of broiler chickens diets with LO and FO alleviate the negative effects of *E. tenella* infection on hematological parameters (RBCs, Hb, PCV, MCV and MCHC).

**Score of Gross Lesions**

In the present study the cecal gross lesions of the examined experimental chickens were recorded in sacrificed chickens at 7th postinfection with sporulated *E. tenella*. The gross lesions scores were assessed using the criteria of Johnson and Reid (1970). Cecal gross lesions were scored from 0 (no gross lesion) and +4 (most severe gross lesions) as shown in Figures 2 and 3. Statistical analysis of gross lesions scores in the different experimental groups is present in Table 5. There were no gross lesions or parasitic density in ceci of the chickens fed diet supplemented with corn oil and non-infected with *E. tenella* (negative control). Whereas, the ceci of chickens fed diet supplemented with 3%
corn oil and infected with *E. tenella* (positive control) showed distention with caseous core. However, the mean lesion scores and parasitic density of the ceci of sacrificed chickens fed diets supplemented with 5% fish oil or 5% linseed oil were significantly lower than other experimental groups. In the broiler chickens fed diets supplemented with LO and FO at 3% the mean lesion scores were also lower than that of the positive control group. With the same concept, Allen *et al.* (1996) found that diets supplemented 2.5 and 10% FO and 10% LO reduced the cecal lesions and degree of mucosal colonization by the development of parasite of broiler chickens infected with *E. tenella*. Those authors attributed these results because of high double bonds of both LO and FO which induce a state of oxidative stress that is detrimental to parasite development.

**Microscopical Examination**

Cecal histopathological lesions were scored from 0-4 as shown in Figures 2 and 3. Significant changes were recorded in scores of histopathological lesions and parasite density
Significantly high scores of cecal histopathological lesions were observed in the chickens fed the control diet supplemented with 3% CO (positive control group) and infected with *E.tenella*. Supplementation the basal diets with 5% FO or 5% LO significantly reduced cecal histopathological lesions scores followed by those fed diets supplemented with 3% FO or LO Table 5. Microscopic examination revealed a striking reduction in the number of parasites seen within the epithelial cells of the caeca in the chickens fed diet supplemented with 5% FO followed by chickens fed 5% LO supplemented diet. It is clear that level of supplemented oil (5 vs 3%) was effective in reduction of parasite density score in the caecal epithelia. However, presence of omega 3 acids was an effective factor Table 5. omega 3 FA are particularly effective against *E.tenella* because the developmental stages, sporulated oocysts and sporozoites, of this *Eimeria* spp. are deficient in superoxide dismutase enzyme, which would protect them from reactive oxygen damage (Abbas *et al.*,...
2012). Feeding broilers with fish oil and flaxseed oil containing diets significantly reduced the degree of parasitization and development of *E. tenella* (Allen *et al.*, 1996) and caused ultrastructural degradation of both asexual and sexual stages, characterized by cytoplasmic vacuolization, chromatin condensation within the nucleus, and lack of parasitophorous vacuole delineation (Danforth *et al.*, 1997). In addition the effects of omega-3 fatty acids (present in FO and LO) may be via the immunomodulatory effect which reduce the effect of inflammation (Korver *et al.*, 1997).

### Cytokines and Total Antioxidant Capacity

Cytokines synthesized and secreted by leukocytes play important regulatory roles during the immune response to infection (Allen and Fetter, 2002). Tumor necrosis factor α (TNF-α) an inflammatory cytokine, is secreted by activated macrophages. Zhang *et al.* (1995) reported that TNF-α-like activity can be detected in stimulated macrophages in sera from infected chickens. There were no significant differences in plasma level of interleukin-1 (IL-1) and tumor necrosis factor (TNF-α) in negative control group and those fed diet supplemented with LO (5%) were higher than other treatment groups. Supplementation of the diet with 5% FO increased plasma level of IL-6 compared with other treatment groups. These results are in agreement with that of Yang *et al.* (2006) who found that chickens supplemented with fish oil (2.5 and 4.5%) and infected with *E. tenella* had higher serum level of IL-6 compared with other groups fed diets supplemented with poultry oil and corn oil. However, those author found that there were no significant differences in serum level of IL-6 and TNF-α in chickens fed diets supplemented with different oils (fish oil, poultry oil, and corn oil) and infected with *E. tenella* at 7 days post infection. Also, the authors suggested that IL-6 contributed to improve the immune responses to avian *E. tenella* infection. Moreover, it has been shown that feeding fish oil supplemented diet in poultry resulted in reduced production of the pro-inflammatory cytokines, IL-1 and TNFα (Korver *et al.*, 1997).

There was a significant increase in serum level of Total Antioxidant Capacity (TAC) of chicken groups fed diets supplemented with 3% and 5% LO and infected with *E. tenella* compared with other groups fed diets.

### Table 5: Gross and Histopathological Lesions Scores and Parasite Density Score in Broiler Chickens Fed Diets Supplemented with FO and LO, Infected with *E. tenella* at 21 Days of Age

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control†</th>
<th>Fish Oil %</th>
<th>Linseed Oil %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-ve</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>+ve</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Gross lesions*</td>
<td>0.00±0.0³</td>
<td>2.7±0.1³</td>
<td>1.1±0.1³</td>
</tr>
<tr>
<td>Histopathological lesions</td>
<td>0.00±0.0³</td>
<td>2.65±0.1³</td>
<td>1.03±0.1³</td>
</tr>
<tr>
<td>Parasitic density</td>
<td>0.00±0.0³</td>
<td>2.56±0.1³</td>
<td>2.5±0.1³</td>
</tr>
</tbody>
</table>

Note: *Gross lesion, f: no gross lesion, d&c: Few petechiae in the cecal wall with the presence of normal contents, b: mild thickness of the cecal wall and bloody content, c: moderate thickness with clotted blood, a: expanded purplish cecal containing large clumps of clotted blood and whitish masses. † = Control diets: supplemented with 3% corn oil, +ve broiler chickens infected with *E. tenella*, -ve broiler chickens non-infected with *E. tenella*.
supplemented with CO or FO. Serum level of lipid peroxidation (MDA) was higher in positive control group (chicken infected with *E. tenella* and fed diet supplemented with 3% CO), where there were no significant differences in serum level of MDA between the negative control group and other chicken groups fed diets supplemented with different oils sources at different levels Table 6. MDA is used as a biomarker for radical-induced damage of biological membranes (Day, 1996). Jafari *et al.* (2012) showed that infected broiler chickens with *E. tenella* had a significantly higher level of plasma and erythrocyte MDA in relation to healthy chickens. The higher concentration of MDA in infected chickens could be attributed to lipid peroxidation resulting from increased Reactive-Oxygen Species (ROS). Wood *et al.* (2003) reported that linseed oil contains lignans that belong to a group of the so-called phytoestrogens that are responsible for the regulation of lipid metabolism and for capturing free radicals which initiate processes of lipids peroxidation and the antioxidative activity of phytoestrogens was revealed to be even significantly higher than that of tocopherols.

**CONCLUSION**

In the face of development of drug resistance almost all over the world and drug residues in food, there is an urgent need to take a shift towards alternative ways for the effective and long term control of avian coccidiosis. In view of the obtained results, it could be concluded that supplementation of broiler diets with linseed oil at 3 or 5% improve growth performance, decrease fecal oocysts count and cecal lesions.

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