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n-3 enriched Fish oil diet enhanced intestinal barrier integrity in broilers after *Eimeria* infection

Yuguo Hou Tompkins, Venkata Sesha Reddy Choppa, and Woo Kyun Kim[1]

Department of Poultry Science, University of Georgia, Athens, GA 30602, USA

ABSTRACT Coccidiosis caused by *Eimeria* spp. results in substantial economic losses in the poultry industry. The objective of this study was to investigate the effects of dietary supplementation with n-3 polyunsaturated fatty acids-enriched fish oil on growth performance, intestinal barrier integrity, and intestinal immune response of broilers challenged with *Eimeria* spp. A total of 576 fourteen-day-old broilers were randomly assigned in a completely randomized design with a 3 × 2 factorial arrangement, comprising 2 diets supplemented with either 5% fish oil or 5% soybean oil, and 3 *Eimeria* spp. infection levels: a nonchallenge control, a low dose of *Eimeria* challenge, and a high challenge dose. The results of the study revealed significant interactions between diet and *Eimeria* challenge to parameters of gut barrier integrity and feed intake. A significant interaction was observed in feed intake between 5 and 8 d postinfection (**DPI**), where the fish oil groups exhibited a higher amount of feed intake compared to the soybean oil diet groups after coccidiosis infection. The effects of the fish oil diet resulted in enhanced gut barrier integrity, as evidenced by a trend of decreased gastrointestinal leakage and a lower mean of small intestine lesion scores after *Eimeria* challenge. Additionally, significant interactions were noted between *Eimeria* spp. challenge and diet regarding jejunal crypt depth. The positive impact of the fish oil diet was particularly noticeable with the high *Eimeria* challenge dose. Overall, these findings underscore the relationship between the fish oil diet and *Eimeria* challenge on broiler chicken intestinal health. Dietary supplementation of fish oil has the potential to maintain small intestine barrier integrity with severe *Eimeria* infection conditions.

Key words: fish oil, n-3 PUFA, coccidiosis, *Eimeria*, intestinal integrity

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INTRODUCTION

With a market preference for drug-free poultry production, the gastrointestinal immune system is constantly challenged by infections caused by intestinal pathogens (Smith, 2011; Gaucher et al., 2015). Coccidiosis, a costly parasitic disease in the broiler industry, is caused by *Eimeria* spp. infection and results in intestinal damage (Dalloul and Lillehoj, 2006; Blake et al., 2020). *Eimeria* infection can interfere with poultry health by causing gut leakage, triggering an immune response, and leading to severe oxidative stress and malnutrition that significantly suppresses growth and production during infection (Choi and Kim, 2022; Sharma et al., 2022; Tompkins et al., 2022).

Fish oil, which is rich in n-3 polyunsaturated fatty acids (**PUFA**), is widely accepted as a nutritional supplement in human studies (Elkin and Harvatine, 2023; Seethaler et al., 2023). The 2 primary bioactive n-3 PUFAs in fish oil are eicosapentaenoic acid (**EPA**; 20:5 n-3) and docosahexaenoic acid (**DHA**; 22:6 n-3) (Alagawany et al., 2019). It has become increasingly clear that dietary long chain n-3 PUFAs can modulate immune status, influencing both humoral and cellular responses in monogastric species (Fritsche and Cassity, 1992; Swiatkiewicz et al., 2015; Al-Khalaifah, 2020; Thanabalan and Kiarie, 2021). Long-chain n-3 PUFAs exhibit natural anti-inflammatory properties as they can be incorporated into membranes of intestinal epithelial cells (Calder 2006; Kalinski, 2012; Xiang et al., 2016). They can reduce the production of pro-inflammatory cytokines and induce the production of anti-inflammatory factors (Calder, 2006; Durkin et al., 2021). The supplementation of fish oil to control intestinal inflammation can promote intestinal health and protect the intestinal mucosal barrier under hostile conditions, which is crucial for maintaining efficient growth performance while defending against pathogenic conditions (Lauridsen, 2020; Tarradas et al., 2020; Durkin et al., 2021). DHA supplementation has been shown to ease

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[1]Corresponding author: wkkim@uga.edu

the severity of necrosis in a rat challenge model (Lu et al., 2007). In pigs, EPA and DHA have been demonstrated to improve intestinal barrier function in weaned pigs after lipopolysaccharide challenges (Zhu et al., 2016). Studies in poultry have shown that 5.0% fish oil adversely affected broilers' performance but exhibited improvement in gut health at the finish stage of growth (Agboola et al., 2021). A diet enriched with n-3 PUFAs can significantly reduce cecal lesions caused by *Eimeria tenella* in broilers (Allen et al., 1996; Aziza et al., 2016; Barua et al., 2016). The reduction of the cecal lesion was linked to the decline in parasite colonization and the inhibition of coccidia development (Allen et al., 1996; Danforth et al., 1997; Allen and Danforth, 1998; Barua et al., 2016). The pro-oxidative nature of n-3 PUFAs plays a significant role in controlling coccidia development, as the incorporation of n-3 PUFAs into both host and parasite membranes altered the stability of the environment for the parasite, making it more vulnerable and decreasing its ability to develop further (Danforth et al., 1997). With a deeper understanding of the benefits of fish oil as a dietary supplement, fish oil supplements have been shown to have protective effects on growth performance and gut health, especially in broilers with present a high immune response to defend against coccidiosis (Barua et al., 2016). Although there have been some inconsistencies in the role of fish oil on broiler gut health with *Eimeria* spp. infection (Yang et al., 2006), most studies have concluded that the dietary supplementation of n-3 PUFAs can be a potential dietary anticoccidial strategy in poultry production to combat coccidiosis (Allen et al., 1996; Danforth et al., 1997; Korver et al., 1997; Allen and Danforth, 1998; Barua et al., 2016). Therefore, due to the anti-inflammatory properties of fish oil in gut health, the current study focused on the impact of n-3 PUFAs supplementation on broiler growth and gut integrity during *Eimeria* infection.

MATERIALS AND METHODS

Ethics Statement

Following the guidelines of the Institutional Animal Care and Use Committee (**IACUC**) at the University of Georgia, the study was conducted at the Poultry Research Center, University of Georgia, Athens, GA after receiving the necessary approvals from IACUC.

Experimental Design

The experiment followed a 2 × 3 factorial design, with diet and infection dose as the main effects. Before birds were allocated into different treatment groups, broiler chicks were fed the same standard starter diet for 0 to 13 d of age. A total of 576 fourteen-day-old male Cobb500 (Cleveland, GA) broiler chickens were randomly assigned to 6 treatment groups, with 8 replicates and 12 birds per cage. There were 2 diet variables: a 5% fish oil diet (Virginia Prime Gold, Omega Protein; Houston, TX) and a 5% soybean oil diet (Harvest Value, US Foods; Austin, TX). Oil was added at the feed mill after all dry ingredients had been mixed, blending for 6 min to eliminate lumps. The *Eimeria* oocysts were orally gavaged at 14 d posthatch, 3 *Eimeria* challenge levels including a nonchallenge control that broilers were gavaged with water, a low challenge dose of *Eimeria spp.* that broilers were gavaged with 12,500 oocysts of *E. maxima*; 12,500 oocysts of *E. tenella*; 62,500 oocysts of *E. acervuline*, a high dose of challenge group that broilers were gavaged with 50,000 oocysts of *E. maxima*, 50,000 oocysts of *E. tenella*, and 250,000 oocysts of *E. acervuline*. The fatty acid profiles of fish oil and soybean oil are shown in Table 1. The experimental groups were set as follows: SO, uninfected broilers fed soybean oil diet; FO, uninfected broilers fed fish oil diet; SO+Low: a low dose of *Eimeria*-infected group fed a soybean oil diet; FO+Low: a low dose of *Eimeria*-infected group fed a fish oil diet; SO+High: a high dose of *Eimeria*-infected group fed soybean oil diet; and FO+High: a high dose of *Eimeria*-infected group fed fish oil diet. All chicks were raised under the same environmental management conditions based on the Cobb500 broiler management guide (Cobb, 2019). Both the starter (1−13 d of age) and the grower (14−26 d of age, treatment diets) diets were formulated according to the Cobb500 broiler management guide, and water and feed were consumed on an ad libitum basis (diet information is shown in Table 2).

Table 1. Fatty acids profiles of fish oil and soybean oil used in current study.

Fatty acids	Area of total fatty acids (%)	
	Fish oil[1]	Soybean oil[2]
Myristic, C14:0	08.90	0.1
Palmitic, C16:0	18.28	10.30
Palmitoleic, C16:1	12.51	5.20
Stearic, C18:0	03.08	3.80
Oleic, C18:1	09.07	22.80
Linoleic, C18:2 (omega-6)	01.41	51.00
Alpha Linolenic, C18:3 (omega-3)	01.36	6.80
Stearidonic, C18:4 (omega-3)	02.81	-
Arachidonic, C20:4 (omega-6)	01.07	-
Eicosapentaenoic, C20:5 (omega-3)	14.67	-
Docosapentaenoic, C22:5 (omega-3)	02.50	-
Docosahexaenoic, C22:6 (omega-3)	11.51	-
Other	12.81	-

[1]Fish oil: Virginia Prime Gold, Omega Protein. It is a refined long-chain omega-3 fish oil specifically formulated as a palatable feed ingredient. Fish oil contains approximately 35% total omega-3 fatty acids with a balanced concentration of EPA and DHA.

[2]Soybean oil: Harvest value, USA. The nutrition facts shown a total saturated fat content is 11%. The soybean oil profile is referred to previous publications (Clemente and Cahoon, 2009; Oladiji et al., 2009).

Growth Performance

At 14 d of age, the body weight (**BW**) of the birds was recorded, and this was considered as 0 d postinoculation (**DPI**). The BW was recorded again at 6 DPI and 12 DPI. The body weight gain (**BWG**) was calculated based on the difference between the initial and final BW. The cumulative feed intake (**FI**) was also recorded, and the feed conversion ratio (**FCR**) was calculated by dividing the amount of feed consumed by the BWG. The FCR was calculated for 2 periods, 0 to 6 DPI and 7

Table 2. Composition and calculated contents of the experimental diets.

		Grower (14–26 d)	
Ingredient (%)	Starter (0–13 d)	Soybean oil diet (SO)	Fish oil diet (FO)
Corn	58.17	55.41	55.41
Soybean meal -48%	34.08	30.44	30.44
Soybean oil	2.00	5.00	0
Fish oil	0	0	5.00
Limestone	0.50	0.47	0.47
Dical. Phos.	1.80	1.70	1.70
Common salt	0.23	0.22	0.22
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.08	0.08	0.08
DL-methionine	0.28	0.25	0.25
L-lysine-HCL	0.16	0.13	0.13
Threonine	0.05	0.04	0.04
Sand	1.00	6.01	6.01
Energy and nutrient composition			
ME, kcal/kg	3008	3086	3086
Crude protein %	21.00	19.00	19.00
Lysine %	1.18	1.05	1.05
Methionine %	0.59	0.54	0.54
Arginine %	1.36	1.23	1.23
Threonine %	0.77	0.69	0.69
Tryptophan %	0.25	0.22	0.22
Valine %	1.03	0.94	0.94
Total sulfur amino acid %	0.88	0.80	0.80
Ca %	0.90	0.84	0.84
Available P %	0.45	0.42	0.42

[1]Vitamin premix include provides the following per kg of diet: Vitamin A 2,204,586 IU, Vitamin D3 200,000 ICU, Vitamin E 2,000 IU, Vitamin B12 2 mg, Biotin 20 mg, Menadione 200 mg, Thiamine 400 mg, Riboflavin 800 mg, d-Pantothenic Acid 2,000 mg, Vitamine B6 400 mg, Niacin 8,000 mg, Folic Acid 100 mg, Choline 34,720 mg.

[2]Mineral premix provides the following per kg of diet: Ca 0.72 g, Mn 3.04 g, Zn 2.43 g, Mg 0.61 g, Fe 0.59 g, Cu 22.68 g, I 22.68 g, Se 9.07 g.

to 12 DPI. Any mortalities that occurred were recorded and taken into account in the FCR calculation.

Gut Permeability

The gut permeability was measured in birds on 5 DPI using a fluorescein isothiocyanate dextran (**FITC-d**) method as previously described (Teng et al., 2020a, 2020b). Briefly, FITC-d (MW 4000; Sigma-Aldrich, Ontario, Canada) was dissolved in distilled water to make the 2.2 mg/mL stock solution. One bird per cage was randomly selected and given 1 mL of FITC-d solution (2.2 mg FITC-d per bird). After 2 h, blood samples were collected from birds and kept in the dark at room temperature and allowed to coagulate, then centrifuged for 15 min at 1,500 *g* to collect serum. For making the standard curve solution, the dilution buffer was made from a pooled serum of noninfected birds on a soybean oil basal diet, and the FITC-d stock solution was serially diluted. The sample and standard solutions were loaded into a black 96-well plate (Corning, Corning, NY), and the FITC-d concentrations were measured using a spectrophotometer (SpectraMax M5; Molecular Devices, San Jose, CA) with the excitation wavelength set at 485 nm and the emission wavelength at 528 nm.

Intestine Lesion Score

At 6 DPI, 3 birds per cage were randomly selected and euthanized by cervical dislocation, then lesion scored by a single evaluator blinded to treatment. The intestine lesion scores were evaluated using a 4-point score scale (Johnson and Reid, 1970), where 0 represents no visible gross lesions, scores greater than zero were considered positive for coccidiosis, and 4 scored as the most severe lesions. The duodenum, a section of the middle intestine (comprising the jejunum and ileum), and ceca were collected for scoring (Conway and McKenzie, 2007). Each section of the small intestine was scored independently, and the average score of each section was calculated and analyzed individually.

Intestine Morphology

At 6 and 12 DPI, one bird per cage was randomly selected to collect intestine tissue samples (8 birds per treatment per time point). Around 3 cm of tissues were dissected from the midpoint of the duodenum, jejunum, and ileum of 1 bird per cage, and then the tissues were rinsed with phosphate-buffered saline and immediately fixed in a 10% neutral buffered formalin solution. The fixed tissues were transported to the Poultry Diagnostic and Research Center (University of Georgia, Athens, GA), where they were further processed. Briefly, the intestinal tissues were dehydrated with increasing concentrations of ethyl alcohol, cleared with xylene, and embedded in paraffin. The tissues were then cut into 4 mm slides using a rotary microtome (Leitz 1512, Leitz, Wetzlar, Germany). The slides were stained with hematoxylin and eosin (**H&E**) and examined under a light microscope using a 4 × objective (Keyence bz-x8000,

Keyence Corp., Osaka, Japan). Representative fields were photographed, and digital images were captured for morphometric analysis. The intestinal histomorphology traits, including mucosa villi height, crypts depth, and villi width, were measured. The villi height was measured from the tip of each villus to the bottom, excluding the intestinal crypts. The villi apical width was measured from the middle part of the villi. Five measurements of the villi height, crypt depth, and villi apical width of the mucosa were conducted on optimally situated samples using the ImageJ software (National Institutes of Health, Bethesda, MD), and the mean value was calculated for statistical analysis. The ratio of villus height to crypt depth was calculated for each field, and the mean ratio of each sample was used for statistical analysis.

Total Antioxidant Status Assay

The total antioxidant capacity of chicken serum was analyzed using QuantiChrom antioxidant assay kits (BioAssay Systems, Hayward, CA). At 6 DPI, one bird per cage was randomly selected for blood collection. The blood samples were collected, allowed to coagulate, and then centrifuged to separate the serum. The serum samples were stored at −80°C until they were needed for the assay, which was performed within a month of sample collection and followed the manufacturer's protocols. The protein concentration was measured using a protein quantification assay (Pierce BCA Protein Assay Kit, Thermo Scientific, Rockford, IL) following the manufacturer's protocols.

Real-Time Quantitative PCR Analysis of Gene Expression

At 6 DPI, 1 bird per replicate was randomly selected for tissue sampling. Cecal tonsil and spleen were snap-frozen by liquid nitrogen and immediately stored at −80°C until RNA isolation. Total RNA was extracted from tissues using Qiazol reagent (Qiagen, Valencia, CA) following the manufacturer's instructions. The quantity of RNA was determined using a Nano-Drop 1,000 Spectrophotometer (ThermoFisher Scientific, Pittsburgh, PA). cDNA was synthesized from the total RNA (2,000 ng) using High-Capacity cDNA Reverse Transcription Kits (Thermo Fisher Scientific, Waltham, MA).

A quantitative real-time reverse transcription polymerase chain reaction (**qRT-PCR**) was performed to quantify the expression levels of mRNA. The primers (Table 3) used in the experiment were designed using the primer design platform Primer-BLAST program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), and primers were validated for specificity based on melting curve and agarose gel electrophoresis analyses. The qRT-PCR was performed using Applied Biosystems StepOnePlus kits (Thermo Fisher Scientific, Waltham, MA) with iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA). The PCR conditions were as follows: initial denaturation at 95°C for 10 min followed by 40 cycles of 15 s at 95°C, 20 s at the annealing temperature, and 1 min at 72°C for extension.

The geometric mean of 18S ribosomal 1(*RNA18S1*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used for normalization (Vandesompele et al., 2002). The stability of the housekeeping genes was confirmed by consistent Ct values among the treatments and duplicates (Stephens et al., 2011). In the cecal tonsils and spleen, the expression of interleukin 10 (*IL10*), interleukin 1 beta (***IL1B***), nuclear factor kappa B subunit 1 (***NFKB1***), chicken tumor necrosis factor-like (***TNF***), and interferon-gamma (***IFNG***) were measured to evaluate the immune responses. Detailed primer sequences used in the experiment are listed in Table 3. Samples were run in duplicates, and relative mRNA expression levels were analyzed using the $2^{-\Delta\Delta Ct}$. The mean ΔCt of each marker gene from the non-infected soybean oil diet (**SO**) was used to calculate the ΔΔCt value, and expression levels were normalized to 1 for SO. The treatment groups' expression levels were presented as fold changes.

Table 3. Nucleotide sequences of the primers used for real-time RT-PCR.

Gene[1]	Primer sequence (5′-3′)	Product length (bp)	Annealing temperature (°C)	Accession #
GAPDH	F-GCTAAGGCTGTGGGGAAAGT R-TCAGCAGCAGCCTTCACTAC	161	55	NM_204305.1
RNA18S1	F-AGCCTGCGGCTTAATTTGAC R-CAACTAAGAACGGCCATGCA	121	56.5	AF_173612.1
NFKB1	F-GAAGGAATCGTACCGGGAACA R-CTCAGAGGGCCTTGTGACAGTAA	131	59	XM_015285418.2
IL10	F-CATGCTGCTGGGCCTGAA R- CGTCTCCTTGATCTGCTTGATG	94	61	NM_001004414.4
IL1B	F-AGATGAAGCGGGTCAGCTC R-GCATCAAGGGCTACAAGCTC	120	59	XM_015297469.2
TNF	F-CGTGGTTCGAGTCGCTGTAT R-CCGTGCAGGTCGAGGTAC	100	60	XM_040694846.2
IFNG	F- AACCTTCCTGATGGCGTGAA R- GCTTTGCGCTGGATTCTCAA	86	60	NM_205149.2

[1]GAPDH: glyceraldehyde-3-phosphate dehydrogenase; RNA18S1: RNA, 18S ribosomal 1; NFKB1: nuclear factor kappa B subunit 1; IL10: interleukin 10; IL1B: interleukin 1 beta; TNF: chTNF-α, chicken tumor necrosis factor-like; INFG: interferon gamma.

Statistical Analysis

All experimental data were tested for normality and homoscedasticity of the residuals. The data were presented as the mean with the standard error of the means (**SEM**). A 2-way ANOVA was used to analyze the means, and the main effects (diets and challenge doses) and their interactions were considered. Tukey's test was further conducted to test differences among the sample means. Generalized linear mixed model (**GLMM**) with the cumulative logit link to statistically analyze coccidial lesion scores (Kang et al., 2019). All statistical procedures were conducted using JMP Pro14 and JMP Add-Ins statistical packages (SAS Institute, Inc., Cary, NC). Statistical significance was set at $P \leq 0.050$, and trends toward statistical significance ($0.050 < P < 0.100$) were also presented in the report (Thiese et al., 2016).

RESULTS

Daily Feed Intake and Growth Performance

The mortality rate was less than 1% in each treatment group. The dietary variable and the *Eimeria* infection independently impacted the daily feed intake. The daily feed intake (**FI**) began to drop significantly from 4 d postinoculation (**DPI**) after the *Eimeria* challenge. The suppression of FI continued throughout the experiment (*P*-challenge < 0.050), with the high inoculation dose leading to an additional reduction in daily feed intake. FO diet groups with a higher FI were observed at 1, 5, 6, and 7 DPI compared to SO groups regardless of *Eimeria*-challenged conditions or doses (*P*-diet < 0.050), while there was no difference between the diet treatments at the other time points. A significant interaction between diet variables and *Eimeria* infection was observed from DPI 5 to DPI 8 (Table 4; *P*-interaction < 0.050). As such, at DPI 6, the low dose of *Eimeria* challenge reduced FI by 68% with the SO diet, and decreased FI by 29% with the FO diet ($P < 0.001$); the high dose of *Eimeria* infection had a much more severe impact on FI. Compared to non-challenge control, the high dose of *Eimeria* challenge reduced FI by 94% with the SO diet, and by 44% with the FO diet ($P < 0.001$). Notably, at DPI 5, 7, and 8, the positive impact of FO on FI was only exhibited under the condition of the high-challenge dose but not with the low-challenge dose of *Eimeria* spp. or control condition. Moreover, the FO diet significantly increased FI compared to the SO diet under the high-challenge conditions ($P < 0.001$), but only numerically increased FI under the low-challenge conditions ($P > 0.05$).

After inoculation, neither cumulative feed intake **(FI),** body weight (**BW**), body weight gain (**BWG**), nor feed conversion ratio (**FCR**) showed any interaction effect between diet and *Eimeria* infection (Table 5; $P > 0.050$). The results underscore a significant growth suppression among broiler chickens following *Eimeria* infection, as evidenced by lower **BW** ($P < 0.001$), reduced **FI** ($P < 0.050$), lower **BWG** ($P < 0.001$), and worsened **FCR** ($P < 0.001$) throughout different study periods, when compared to non-challenged groups. A notable trend showed that the higher *Eimeria* challenge doses exacerbated the negative impact on growth parameters. The diet effect, as another main factor, significantly impacted FI, with FO groups showing higher FI compared to SO groups ($P < 0.050$) during 0-6 DPI and 0-12 DPI study periods, but not during 7-12 DPI ($P > 0.050$). In terms of growth parameters, the incorporation of FO led to a 7.3 % increase in BWG compared to SO groups during DPI 0-6 ($P = 0.071$), and a numerically higher BW at DPI 6 ($P = 0.071$). However, the dietary treatments did not affect BWG during DPI 7-12 ($P > 0.050$) or over the total study period from DPI 0 to DPI12 ($P >$

Table 4. Daily feed intake (g) from 0 d postinoculation (**DPI**) to 12 DPI.

Treatments[1]		DPI 1	DPI 2	DPI 3	DPI 4	DPI 5	DPI 6	DPI 7	DPI 8	DPI 9	DPI 10	DPI 11	DPI 12
SO		77.9	68.4	82.4	77.9	71.4 a	101.2[a]	100.5[a]	107.0[a]	115.9	120.1	128.7	99.8
FO		82.2	71.5	84.4	80.7	69.5[a]	101.3[a]	104.4[a]	105.1[a]	116.3	129.8	133.5	100.5
SO+Low challenge		78.4	68.1	83.8	68.8	49.7[bc]	60.0[cd]	80.7[bc]	95.0[ab]	104.1	115.9	115.5	87.4
FO+Low challenge		82.1	68.0	81.4	67.7	53.9[b]	78.1[b]	83.6[bc]	97.2[ab]	97.1	116.7	114.4	83.3
SO+High challenge		81.4	67.2	81.5	58.5	36.4[d]	52.6[d]	72.6[c]	89.2[b]	94.7	109.6	112.9	85.2
FO+High challenge		84.0	68.6	80.6	59.2	47.1[c]	70.3[bc]	85.6[b]	101.5[a]	97.9	114.3	110.3	86.2
SEM		0.692	0.568	0.557	1.344	1.898	2.937	2.111	1.573	1.664	1.606	1.719	1.427
Means of main effect													
Diet	SO	79.7[b]	67.9	82.6	68.4	52.5	71.3	85.7	97.1	104.9	115.2	119.0	90.9
	FO	83.0[a]	69.4	82.2	69.2	56.8	83.3	91.2	101.3	103.7	120.3	119.4	90.7
Challenge	NC	80.7	69.9	83.40	79.3[a]	70.5	101.3	103.1	106.0	116.1[a]	124.9[a]	131.1[a]	101.7[a]
	Low	80.3	68.1	82.6	68.3[ab]	51.8	69.1	82.2	96.1	100.6[b]	116.3[ab]	114.9[b]	85.3[b]
	High	83.1	67.9	81.0	58.8[c]	41.7	61.4	79.1	95.3	96.3[b]	112.0[b]	111.6[b]	85.4[b]
Source of Variance *P*-value													
P-Diet		0.010	0.207	0.712	0.486	0.001	< 0.001	0.008	0.134	0.600	0.077	0.880	0.906
P-Challenge		0.136	0.282	0.215	< 0.001	< 0.001	< 0.001	< 0.001	0.0040	< 0.001	0.006	< 0.001	< 0.001
Interaction *P*-value													
Challenge*Diet		0.970	0.530	0.261	0.430	< 0.001	0.002	0.048	0.042	0.181	0.932	0.391	0.132

[a,b,c]Mean values with different superscript letters within the same column indicate a significant difference ($P \leq 0.05$), N = 8.

[1]SO, negative control with soybean oil diet; FO, negative control with fish oil diet; SO+Low challenge, soybean oil diet group that challenge with low dose of *Eimeria* spp. solution; FO+Low challenge, fish oil diet group that challenge with low dose of *Eimeria* solution; SO+High challenge, soybean oil diet group that challenge with high dose of *Eimeria* solution; FO+High challenge, fish oil diet group that challenge with high dose of *Eimeria* solution.

Table 5. Cumulative feed intake (**FI**), body weight gain (**BWG**) and feed conversion ratio (**FCR**) from 0 d postinoculation (**DPI**) to 12 DPI.

		BW (g)		DPI 0–6			DPI 7–12			DPI 0–12		
Treatmemts[1]	DPI0	DPI6	DPI12	FI (g)	BWG (g)	FCR	FI (g)	BWG (g)	FCR	FI (g)	BWG (g)	FCR
SO	331.0	623.6	1067.8	480.7	292.5	0.608	675.9	444.2	0.657	1,156.6	736.7	0.638
FO	331.8	639.2	1103.7	489.6	307.3	0.627	692.0	464.5	0.668	1,181.7	771.9	0.652
SO+Low challenge	331.7	545.8	920.5	408.8	214.1	0.523	598.5	374.7	0.623	1,007.4	588.8	0.584
FO+Low challenge	331.0	568.5	908.0	431.3	237.5	0.550	592.2	339.4	0.571	1,023.5	577.0	0.563
SO+High challenge	330.4	512.7	833.1	377.6	182.4	0.444	564.0	320.4	0.566	941.5	502.7	0.533
FO+High challenge	328.6	522.9	884.6	410.6	194.3	0.471	595.5	361.7	0.605	1,006.0	556.0	0.552
SEM	0.505	7.867	16.570	6.338	8.197	0.013	8.352	11.077	0.013	13.777	16.487	0.009
Means of main effect												
Diet SO		560.7	940.5	422.3[b]	229.7	0.525	612.8	379.8	0.616	1,035.1[b]	609.4	0.585
FO		576.9	965.4	443.8[a]	246.4	0.550	626.6	388.5	0.615	1,070.4[a]	634.9	0.589
Challenge NC		631.4[a]	1085.7[a]	485.2[a]	299.9[a]	0.618[a]	684.0[a]	454.4[a]	0.662[a]	1,169.1[a]	754.3[a]	0.645[a]
Low		557.2[b]	914.2[b]	420.0[b]	225.8[b]	0.537[b]	595.3[b]	357.1[b]	0.600[ab]	1,015.4[b]	582.9[b]	0.573[b]
High		517.8[c]	858.9[c]	396.1[c]	188.3[c]	0.458[c]	579.7[b]	341.0[b]	0.586[b]	973.8[c]	529.4[c]	0.543[b]
Source of variation *P*-value												
Diet		0.088	0.162	<0.001	0.071	0.115	0.178	0.642	0.975	0.005	0.153	0.746
Challenge		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.03	<0.001	<0.001	<0.001
Interaction *P*-value												
Challenge*diet		0.858	0.308	0.198	0.865	0.972	0.315	0.238	0.318	0.221	0.304	0.386

[a,b,c]Mean values with different superscript letters within the same column indicate a significant difference ($P \leq 0.05$), N = 8.

[1]SO, negative control with soybean oil diet; FO, negative control with fish oil diet; SO+Low challenge, soybean oil diet group that challenge with a low dose of *Eimeria* spp. solution; FO+Low challenge, fish oil diet group that challenge with a low dose of *Eimeria* solution; SO+High challenge, soybean oil diet group that challenge with a high dose of *Eimeria* solution; FO+High challenge, fish oil diet group that challenge with a high dose of *Eimeria* solution.

0.050). The FO supplementation did not influence FCR at any time point during the study.

Gastrointestinal Permeability, Lesion Score, and Intestinal Morphology

At 5 DPI, the gastrointestinal leakage studies suggested a trend of interactive effect ($P = 0.055$) between diet and challenge. The significantly increased serum concentrations of fluorescein isothiocyanate dextran (**FITC-d**; μg/mL) revealed intestinal leakage with *Eimeria* spp. challenge ($P < 0.001$; Figure 1A). In terms of dietary treatments, FO groups exhibited lower concentrations of FITC-d in the serum compared to SO groups ($P = 0.001$; Figure 1B), suggesting less intestinal damage with FO supplementation.

At 6 DPI, lesion score readings showed no lesions in the intestines of birds from the unchallenged negative control groups (Figure 2). The lesion score results indicated a significant interaction between challenge and diet treatments in the jejunum and ileum ($P = 0.050$), in the duodenum ($P = 0.037$), and in the ceca ($P = 0.050$). Notably, the beneficial impact of FO in decreasing small intestinal lesions was evident solely under conditions of high doses of *Eimeria* challenge ($P < 0.050$). In regards to main effects, *Eimeria* infection significantly impacted the severity of intestinal lesions in the duodenum ($P < 0.050$), jejunum/ileum ($P < 0.050$), and ceca ($P < 0.050$) compared to non-challenged control groups, while FO diet reduced the lesion score mean in the jejunum/ileum ($P < 0.050$) and duodenum ($P < 0.050$), and with a tendency to lower ceca lesion ($P = 0.064$) compared to the SO diets.

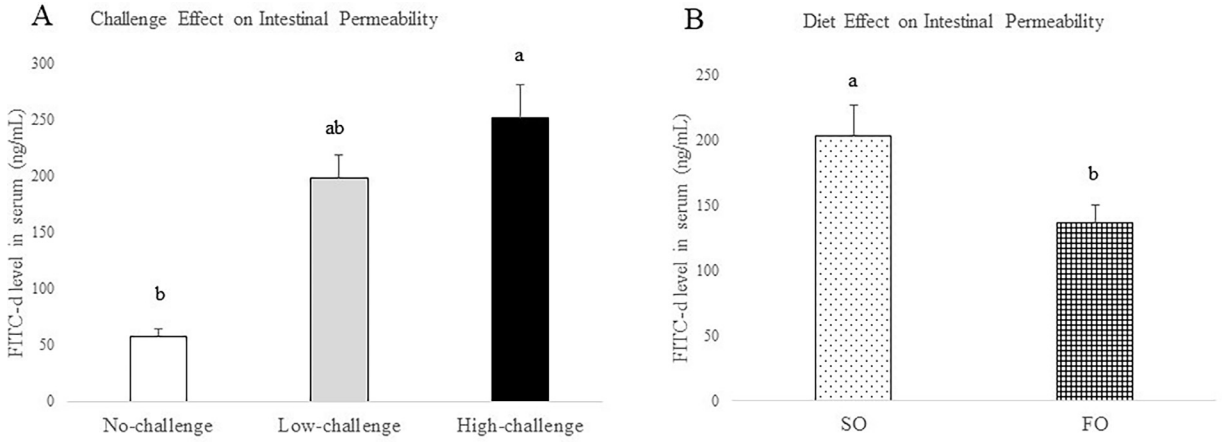

Figure 1. The effect of diet and *Eimeria* spp. challenge on broiler chicken gut permeability at 5 d of postinoculation (**DPI**). SO, groups with soybean oil diet; FO, groups supplemented with fish oil diet; No-challenge, control groups that were not exposed to *Eimeria* spp.; Low-challenge: groups were challenged with a low dose of *Eimeria* spp. solution; High-challenge, groups that were challenged with a high dose of *Eimeria* spp. solution. The error bars represent the SEM values. Bars without a common letter differ significantly ($P \leq 0.05$, N = 8) according to Tukey's HSD test. *P*-values: *P*-challenge < 0.001; *P*-diet= 0.001; *P*-interaction between *Eimeria* spp. challenge and diet = 0.055.

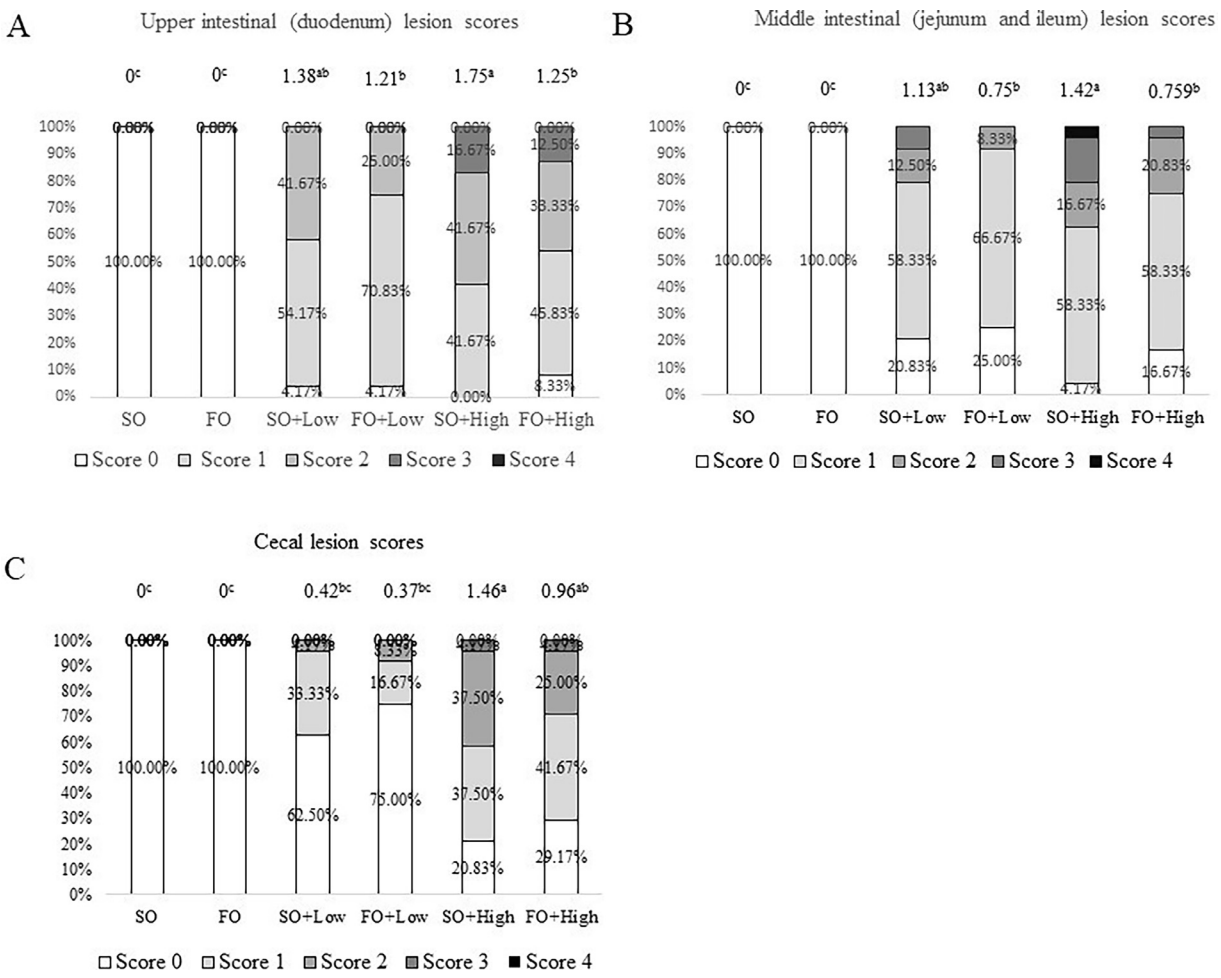

Figure 2. The effect of diet treatments on intestinal lesion scores of broiler chickens subjected to different *Eimeria* spp. inoculation doses at 6 d postinoculation. A 0 represents no visible gross lesions and 4 signifies the most severe lesions. *P*-value: *P*-interaction between the *Eimeria* challenge dose and diet in the jejunum and ileum = 0.050 (Figure 2A), in the duodenum *P*-interaction = 0.037 (Figure 2B), and in the ceca *P*-interaction = 0.050 (Figure 2C).

SO, negative control with soybean oil diet; FO, negative control group with fish oil diet; SO+Low, soybean oil diet group that was challenged with a low dose of *Eimeria* solution; FO+Low, fish oil diet group was challenged with a low dose of *Eimeria* solution; SO+High, soybean oil diet group was challenged with a high dose of *Eimeria* spp. solution; FO+High, fish oil diet group was challenged with a high dose of *Eimeria* spp. solution. [a–c] Mean values with different superscript letters indicate a significant difference in lesion score means ($P \leq 0.05$).

The examination of intestinal morphology at 6 DPI showed characteristic signs of coccidiosis in broilers challenged with *Eimeria* spp. (Figure 3; Table 6). The duodenum morphology was significantly affected by *Eimeria* infection, causing a reduction in villi height (*P*-challenge< 0.001). A higher inoculation dose led to an additional reduction in villi height. The *Eimeria* infection also increased crypt depth ($P < 0.001$) and decreased villus-to-crypt ratios ($P < 0.001$), but did not affect duodenum villi width ($P > 0.050$). In the jejunum, *Eimeria* infection significantly decreased villi height ($P < 0.001$) and villus-to-crypt ratios ($P < 0.001$), while jejunal villi width remained unaffected ($P > 0.050$). The *Eimeria* infection significantly affected ileum crypt depth ($P < 0.001$) and decreased villus-to-crypt ratios ($P < 0.001$), but did not affect ileum villi height or width ($P > 0.050$). Regarding the diet treatments, the FO diet groups exhibited a narrower villi width ($P = 0.013$) compared to the SO diet groups. Notably, there was a significant interaction between diet variables and *Eimeria* infection in terms of jejunal crypt depth (Table 6; *P*-interaction < 0.050), showing an infection dose-dependent response. Under low *Eimeria* challenge dose conditions, the FO diet tended to increase the depth of the crypts in the jejunum compared to the SO diet. In contrast, under the high-challenge dose of *Eimeria* spp. condition, the FO diet tended to decrease jejunal crypt depth compared to the SO diet. No significant interactions were observed between infectious doses and diets for other morphological characteristics ($P > 0.050$).

Total Antioxidant Capacity

The total antioxidant capacity was significantly affected by *Eimeria* spp. challenge at 6 DPI ($P < 0.050$; Figure 4A), but there was no significant effect of the diet treatments on the total antioxidant capacity. At 12 DPI, neither the challenge effect nor the diet effect

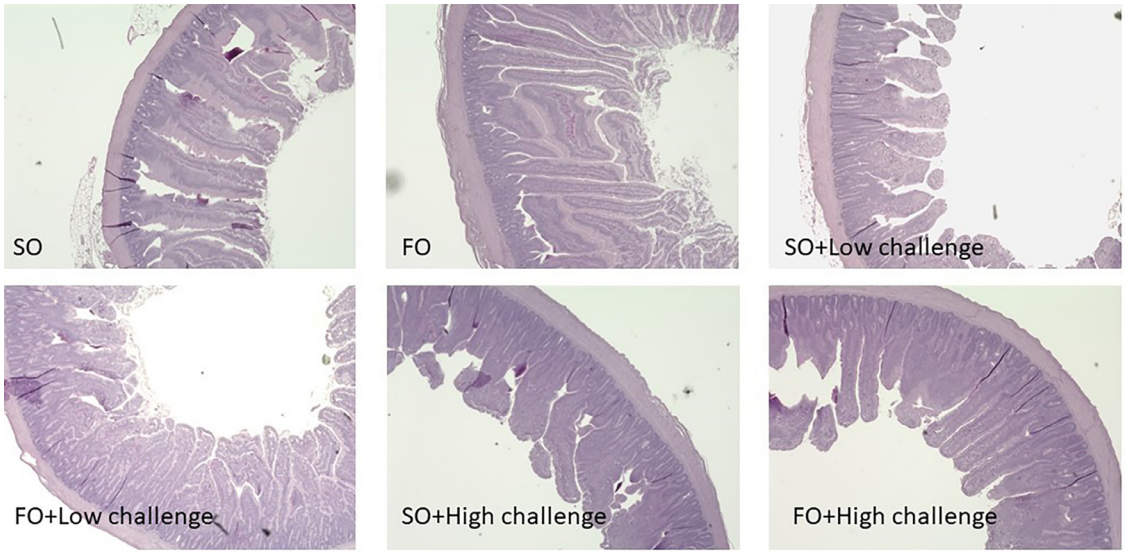

Figure 3. Histological examination of the intestine demonstrated characteristic microscopic signs of coccidiosis in the affected broilers at 6 d postinoculation. SO, negative control with soybean oil diet; FO, negative control with fish oil diet; SO+Low challenge, soybean oil diet group that was inoculated with a low dose of *Eimeria* spp. solution; FO+Low challenge, fish oil diet group that was inoculated with a low dose of *Eimeria* spp. solution; SO+High challenge, soybean oil diet group that was challenged with a high dose of *Eimeria* spp. solution; FO+High challenge, fish oil diet group that was inoculated with a high dose of *Eimeria* spp. solution.

significantly impacted the serum total antioxidant capacity (Figure 4B; $P > 0.050$).

The mRNA Expression of Pro-inflammatory and Anti−inflammatory Cytokines in Cecal Tonsils and Spleen

At 6 DPI, the mRNA expression of *NFKB1, IL10*, and *IL1B* in the cecal tonsils was significantly reduced by *Eimeria* infection compared to the uninfected control groups ($P < 0.05$; Figure 5A), whereas the mRNA expression of *TNF* and *IFNG* remained unchanged with infection at 6 DPI ($P > 0.05$). In terms of dietary variables, the fish oil diet significantly increased *IL10* mRNA expression compared to the soybean oil diet ($P < 0.050$). However, there was no interaction between diet and infection variables on the expression of immune response-related marker genes in the cecal tonsils.

Table 6. The villi height, villi width, crypts depth, and villus/crypt ratio in the duodenum, jejunum, and ileum of broilers at 6 d postinoculation (**DPI**).

Treatment[1]		Duodenum (μm)				Jejunum (μm)				Ileum (μm)			
		V[2]	Vw[3]	C[4]	V:C[5]	V	Vw	C	V:C	V	Vw	C	V:C
SO		710.26	66.28	55.56	14.42	449.93	64.83	63.77^{b}	7.65	209.95	55.26	48.90	4.85
FO		730.98	91.00	56.17	14.11	408.54	45.69	55.38^{b}	7.69	193.48	55.94	38.90	5.17
SO+Low challenge		541.69	58.83	118.44	4.89	302.18	73.54	97.36^{ab}	3.25	219.26	58.76	81.52	2.78
FO+Low challenge		567.65	67.00	108.27	5.52	253.89	56.16	121.702^{a}	2.11	199.69	69.59	75.75	2.72
SO+High challenge		455.98	78.28	121.50	4.30	308.79	82.02	125.31^{a}	2.62	172.44	67.77	84.19	2.19
FO+High challenge		422.94	74.37	135.92	3.56	276.22	60.59	83.58^{ab}	3.85	192.46	68.37	81.65	2.49
SEM		21.99	3.54	6.06	0.83	16.84	3.88	6.31	0.42	5.79	2.32	3.54	0.25
Means for the main effect													
Diet	SO	569.31	67.78	98.50	7.87	353.63	73.46^{a}	95.48	4.50	200.55	60.60	71.54	3.27
	FO	573.86	72.56	100.12	7.73	312.88	54.14^{b}	86.89	4.55	195.21	64.63	65.43	3.46
Challenge	No	720.62^{a}	71.30	55.86^{b}	14.27^{a}	429.23^{a}	55.26	59.58	7.67^{a}	201.71	55.60	43.90^{b}	5.01^{a}
	Low	554.67^{b}	62.91	113.36^{a}	5.21^{b}	278.04^{b}	64.85	109.53	2.68^{b}	209.47	64.18	78.63^{a}	2.75^{b}
	High	439.46^{c}	76.32	128.71^{a}	3.93^{b}	292.50^{b}	71.30	104.45	3.23^{b}	182.45	68.07	82.92^{a}	2.34^{b}
Source of Variation *P*-value													
Diet		0.749	0.315	0.919	0.888	0.977	0.013	0.420	0.9315	0.483	0.486	0.161	0.572
Challenge		< 0.001	0.071	< 0.001	< 0.001	< 0.001	0.213	< 0.001	< 0.001	0.143	0.09	< 0.001	< 0.001
Interaction													
Challenge* Diet		0.680	0.432	0.483	0.850	0.973	0.975	0.047	0.186	0.306	0.573	0.816	0.886

^{a,b,c}Mean values with different superscript letters within the same column indicate a significant difference ($P \leq 0.05$), N = 8.

[1]SO, negative control group with soybean oil diet; FO, negative control group with fish oil diet; SO+Low challenge, soybean oil diet group was challenged with a low dose of *Eimeria* spp. solution; FO+Low challenge, fish oil diet group was challenged with a low dose of *Eimeria* solution; SO+High challenge, soybean oil diet group that was challenged with a high dose of *Eimeria* solution; FO+High challenge, fish oil diet group that was challenged with a high dose of *Eimeria* solution.

[2]V: villi height.

[3]Vw: villi width.

[4]C: crypts depth.

[5]V: C: villus height and crypt depth ratio.

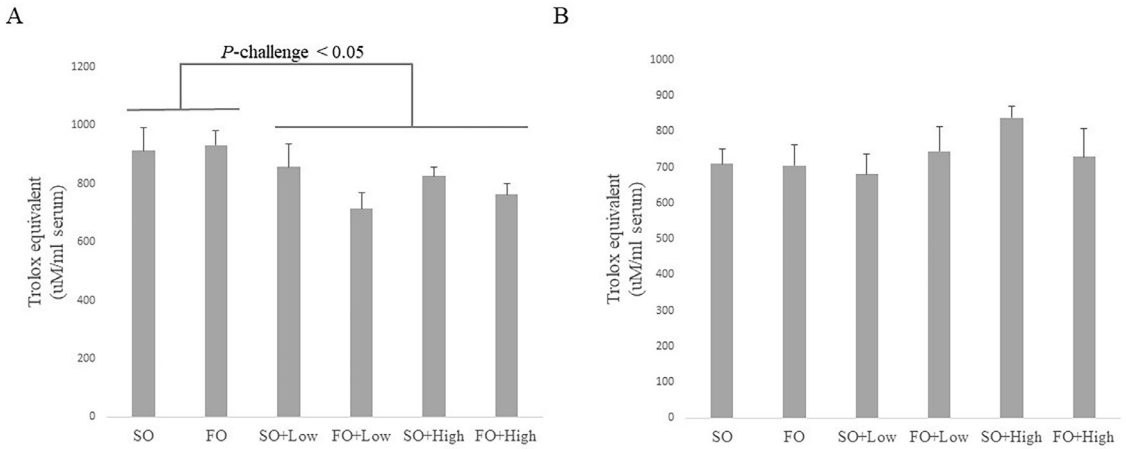

Figure 4. Total antioxidant capacity in serum. (A) The total antioxidant capacity in serum at 6 d postinoculation. *P*-Value: *P*-challenge < 0.050, *P*-diet and *P*-interaction > 0.050, N = 8 (B) The total antioxidant capacity in serum at 12 d postinoculation; *P*-Value: *P*-challenge, *P*-diet and *P*-interaction > 0.050, N = 8.

SO, negative control with soybean oil diet; FO, negative control group with fish oil diet; SO+Low, soybean oil diet group that was inoculated with a low dose of *Eimeria* solution; FO+Low, fish oil diet group was inoculated with a low dose of *Eimeria* solution; SO+High, soybean oil diet group was inoculated with a high dose of *Eimeria* spp. solution; FO+High, fish oil diet group was inoculated with a high dose of *Eimeria* spp. solution.

In spleen, the expression of *IL10* mRNA was observed to be increased in birds infected with *Eimeria* spp. compared to the uninfected control group, although this difference was not statistically significant ($P = 0.072$; Figure 5B). In contrast, there was a significant decrease in the mRNA expression of *TNF* in the high-challenge dose groups compared to the low-infection groups and uninfected control group ($P < 0.050$; Figure 5B).

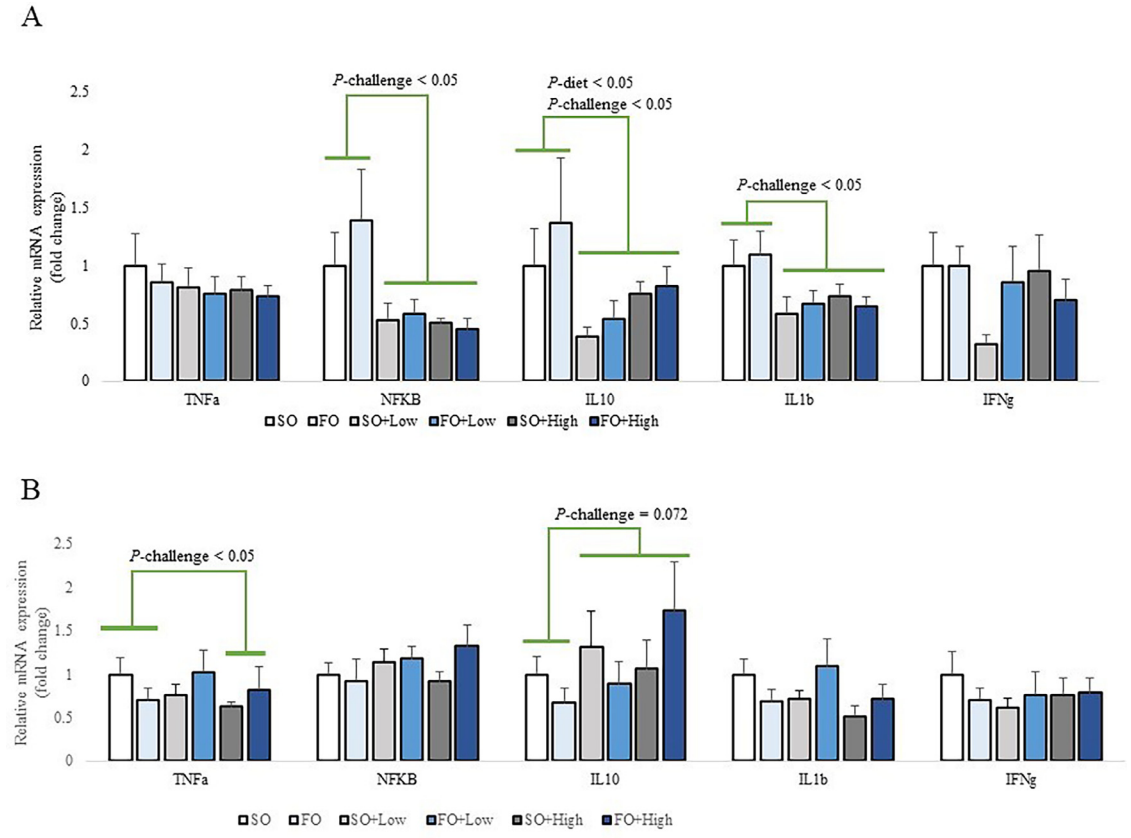

Figure 5. The effect of fish oil diet and *Eimeria* challenge on relative mRNA expression of inflammatory and anti−inflammatory cytokines in the cecal tonsils (A) and the spleen (B) at 6 DPI. There was no interaction between diet and infection variables on the mRNA expression of cytokine markers in the cecal tonsils and spleen. Fold change of relative gene expression ($2^{-\Delta\Delta Ct}$) was calculated using the ΔΔCt method, with the geometric mean of 18S ribosomal 1(*18S*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the endogenous control. SO, negative control with soybean oil diet; FO, negative control group with fish oil diet; SO+Low, soybean oil diet group that was inoculated with a low dose of *Eimeria* solution; FO+Low, fish oil diet group was inoculated with a low dose of *Eimeria* solution; SO+High, soybean oil diet group was inoculated with a high dose of *Eimeria* spp. solution; FO+High, fish oil diet group was inoculated with a high dose of *Eimeria* spp. solution.

However, there was no significant interaction between diet and infection variables on the expression of immune response-related genes in the spleen.

DISCUSSION

The findings of this study and previous studies (Allen et al., 1996; Danforth et al., 1997; Allen and Danforth, 1998; Yang et al., 2006; Agboola et al., 2021) all indicate that fish oil diets can reduce the severity of intestinal lesions caused by *Eimeria* infection, particularly in the jejunum. In recent years, long-chain fatty acids, particularly long-chain n-3 PUFAs, have received increased attention in animal nutrition research due to their health benefits (Thanabalan and Kiarie, 2021). n-3 PUFAs can be incorporated into cell membranes, which affects their antioxidant signaling and helps to regulate oxidative stress levels (Garrel et al., 2012). Studies have shown that the supplementation of a fish oil diet in rats increased the expression and activity of the antioxidant enzyme superoxide dismutase (SOD) and a decrease in plasma membrane peroxidation (Erdogan et al., 2004; Garrel et al., 2012).

Although a few studies have suggested that n-3 PUFAs can exhibit a dual role as pro-oxidants and antioxidants depending on the type of cells being studied (Di Nunzio et al., 2011; Radzikowska et al., 2019), the majority of studies have concluded that adequate dietary supplementation of n-3 PUFAs can improve resistance to free radical attacks and reduce lipid peroxidation, particularly in situations of stress or infection (Oppedisano et al., 2020). The n-3 PUFA-enriched fish oil diet is highly susceptible to oxidation due to the multiple double bonds it contains (Awada et al., 2012). Oxidized lipids are pro-apoptotic factors that can induce cell damage and cause extensive tissue damage that causes losses in productive performance (Awada et al., 2012). It is important to note that the protective effect of antioxidants against oxidative damage in diets enriched with n-3 PUFAs may vary depending on the type and amount of antioxidants added to the diet. Antioxidants in the diet play a protective role in reducing fat oxidation, as well as protecting the tissue from oxidative damage caused by lipid oxidation (Lesson and Summers, 2001). The additional antioxidant content required in poultry diets increases as the content of polyunsaturated oil content increases (Surai, 2007). For example, when soybean oil content increases by 1% in the diet, it is advised to increase vitamin E level by 20 IU, and when fish oil content increases by 1%, the requirement for additional vitamin E level is 8 IU (Lesson and Summers, 2001). Based on the current experiment setting, it is possible that the soybean oil group may have experienced more significant depletion of antioxidants, and that oxidative stress may have contributed to the lower growth observed in birds on the soybean oil diet compared to those on the fish oil diet. However, based on the current data, there were no significant differences observed in the serum total antioxidant capacity between the 2 oil groups. The exact impact of n-3 PUFA-enriched fish oil on antioxidative or prooxidative properties in the presence of *Eimeria* infection is still not clear. and further research is needed to fully understand the mechanisms involved.

It is well-known that eicosapentaenoic acid (**EPA**; 20:5 n-3), docosahexaenoic acid (**DHA**; 22:6 n-3), and arachidonic acid (**AA**, n-6 PUFA) are constituents of the cell membrane, where AA act as a pro-inflammatory factor (Alhusseiny and El-Beshbishi, 2020). The amounts and ratios of these long-chain fatty acids in cell membranes can be altered through dietary supplementation of n-3 PUFAs (Calder, 2013), and n-3 PUFAs have been shown to modulate the immune response, affecting both humoral and cellular responses in human and mice (Erickson, 1986; Radzikowska et al., 2019). Increased consumption of n-3 PUFAs can reduce the amount of AA in cell membranes, change the inflammatory state of cells, and alter the response of immune cells by inhibiting the overproduction of proinflammatory mediators such as proinflammatory cytokines and inflammatory eicosanoids (Calder, 2006; Eilati et al., 2013). Studies have shown that n-3 PUFAs have anti-inflammatory effects on mammal endothelial cells (Radzikowska et al., 2019; Lauridsen, 2020). Chickens possess avian-specific lymphoid organs, including the cecal tonsils and bursa of Fabricius, that serve as part of their gut defense system to combat intestinal pathogens like coccidia (Yun et al., 2000). In the current study, the *Eimeria*-infected groups showed a numerically higher level of *IL10* in the spleen compared to the non-infected control. With regard to diet variables, the expression of *IL10* increased in the cecal tonsils in the fish oil diet group. *IL10* mRNA expression in chickens is primarily found in the bursa of Fabricius and cecal tonsils (Rothwell et al., 2004). *IL10* is a crucial anti-inflammatory cytokine that helps prevent the overproduction of inflammatory factors (Lee et al., 2018; Arendt et al., 2019). However, some previous studies have hypothesized that protozoan parasites can take advantage of the host's IL10 to dampen the chicken immune response in the surrounding microenvironment (Arendt et al., 2019). There have been reports of an increase in the serum or intestine level of *IL10* in response to *Eimeria* infection (Cornelissen et al., 2009; Morris et al., 2015). The suppressing of *Eimeria*-induced *IL10* through the use of antibodies has been shown to reduce performance loss (Sand et al., 2016; Arendt et al., 2019). We hypothesize that the variations in the immune responses' patterns could be due to factors such as the dose administered, genetic components of host species and *Eimeria* species, the tissue types, and the time of sampling. Additionally, the lipid content, level of dietary fat peroxidation, and antioxidant content of the diet may also play a role in affecting the immune response (Fries-Craft et al., 2021). Based on the results of the current study, we conclude that the consumption of fish oil has a positive impact on gut integrity during *Eimeria* infection compared to a diet rich in soybean oil. The production of *IL10* can be a key factor in regulating

the immune response and maintaining gut permeability following *Eimeria* infection when birds consume a fish oil diet. Further research is needed to fully understand the role of n-3 PUFAs under the condition of pathogen challenges.

With a high dose of *Eimeria* spp. inoculation, we frequently observed a significant difference between the FO diet and the SO diet in terms of daily feed intake, gut lesion and gut, and gut permeability. Conversely, when the challenge dosage is low, the impact of the FO diet was less pronounced and mostly showed only a trend, indicating that other than the fish oil dosage, the *Eimeria* challenge doses are another essential factor to consider for experimental design. Previous studies have reported severe gut leakage on 5, 6, and 7 DPI after the *Eimeria* challenge (Teng et al., 2020a), which is attributed to the reproduction and expansion stage of the parasite's life cycle. The duodenum is the primary intestinal compartment responsible for fat digestion, and the jejunum is the main place of fat absorption, with a small portion of fat digestion continuing in the upper ileum (Tancharoenrat et al., 2014; Rodriguez-Sanchez et al., 2019). The improved gut barrier in the duodenum and jejunum enables better absorption and digestion of dietary lipids. In a previous study, it was observed that the supplementation of fish oil could suppress coccidia development, as indicated by the abnormal shedding of asexual and sexual parasites in the cecal lumen (Danforth et al., 1997). Moreover, several investigations have indicated that the cecal tonsils play a crucial role in the intestinal immune response to *Eimeria*, and an increase in the number of $CD4^+$ cecal tonsil lymphocytes was observed on 4 and 6 DPI (Lillehoj and Trout, 1996; Yun et al., 2000). The innate immune response is essential in controlling coccidia development, and nutrient supplementation has been shown to positively affect intestinal health and increase feed intake by modulating the inflammatory status. Furthermore, studies have shown that a diet rich in polyunsaturated fatty acids diet can alter the gut microbiota and has an anti-microbial effect (Warner et al., 2019). Both EPA and DHA have been found to exhibit *in vitro* anti-microbial activities against several species of bacteria (Correia et al., 2012; Desbois and Lawlor, 2013). *Clostridium perfringens* (*C. perfringens*) is an anaerobic, gram-positive bacterium that is associated with acute gastrointestinal infections in poultry (Nicholds et al., 2021). Although *C. perfringens* is commonly present in the intestines of birds, it can overgrow in its favorable environment, such as an *Eimeria* infection, and cause severe necrotic enteritis (Collier et al., 2008; Fathima et al., 2022). Therefore, the reduced loss of growth with fish oil in the current study might be attributed to the antimicrobial effect of fish oil, which suppressed the growth of pathogenic bacteria. This proposition is in agreement with the conclusions from a previous study that short-term fish oil supplementation during the acute infection stage can minimize production losses caused by coccidiosis (Allen et al., 1996). However, it is important to note that a diet containing 5% fish oil may present some difficulties for long-term use, which might be due to the feed cost and the need for proper storage conditions and management. Previous studies have indicated that dietary fish oil supplementation did not significantly impact broiler chicken performance during the starter phase, but negatively affected the final weight in the finisher phase (Agboola et al., 2021). It is crucial to optimize the timing and duration of the FO diet use. In order to mitigate the potential negative impact of the FO diet, we selected the mid-stage of growth as the study period, which aligns with the period of the coccidiosis outbreak in commercial production (Teng et al., 2020a). Based on the current data, it is recommended to supplement broilers with fish oil for a short period during the acute coccidiosis infection stage. However, determining effective doses of n-3 PUFAs remains challenging due to inconsistent results from in vivo and in vitro studies. Further research is needed to determine the exact mechanisms and factors involved, and mostly, identify the most effective dose of n-3 PUFAs for controlling coccidia infection in chickens and to understand the interaction between n-3 PUFAs and *Eimeria*'s life cycle.

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DISCLOSURES

The authors declare no conflicts of interest.

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