Asian Journal of Poultry Science

ISSN 1819-3609

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Poultry Science

ISSN 1819-3609
DOI: 10.3923/ajpsaj.2017.20.30

Research Article
Effect of Fish Oil on Performance, Coccidiosis Prevention and Serum Lipid Profile in Broiler

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Abstract

Background and Objective: Fish Oil (FO) is an important source of omeg-3 polyunsaturated fatty acids (PUFA) which increases performance, immunity to broilers. The present investigation was undertaken to determine the influence of dietary FO on performance, preventing coccidiosis and serum lipid profile in broilers. **Materials and Methods:** A total of 120 commercial chicks were randomly distributed in four groups which were treated either without FO (T_0/control) or with 1% FO (T_1), 2% FO (T_2) and 3% FO (T_3) in regular drinking water. The experiment was divided into three sections. Firstly, production performance of broilers with FO treatment was assigned. Secondly, gross and histo-pathological changes were determined in chicks infected with 10^4 sporulated oocysts of *Eimeria* spp. Finally, changes in different lipid profile parameters in broilers serum were measured. Data analysis was done for ANOVA by STATA version-12.1 where a p-value of $\leq$0.05 or $\leq$0.01 was considered statistically significant. **Results:** It was observed that FO in regular drinking water significantly ($p<0.01$) increased live weight, weight gain, feed consumption at 3rd and 4th weeks of age of broilers which were higher in T_2 and T_3 groups compared to others. Feed Conversion (FC) was significantly ($p<0.01$) better in FO supplemented groups. At necropsy, birds of control, T_1 groups developed severe gross and histopathological lesions of coccidiosis. However, no lesions were developed in birds of T_2 or T_3 groups. With increasing levels of FO in the broiler diets, serum HDL-cholesterol level increased and cholesterol, LDL-cholesterol, triglycerides levels decreased significantly ($p<0.01$). **Conclusion:** It can be concluded that supplementing either 2 or 3% fish oil in regular drinking water of broiler is helpful in increasing performance, improving serum lipid profile of broilers. Birds also represent a high immune response against coccidiosis at these levels. All these positive effects can be gained in broiler if we can establish using fish oil in regular drinking water of broilers. Moreover, there will be least chance of developing drug resistance in broilers eventually in human caused by using coccidiostat for prevention of coccidiosis.

Key words: Fish oil, performance, coccidiosis, histopathology, lipid profile

Received: September 15, 2016 Accepted: November 14, 2016 Published: December 15, 2016

Citation: Mukti Barua, Goutam Buddha Das, Kamrul Islam, Sharmin Chowdhury, Md. Emran Hossain, Babu Kanti Nath and Md. Shafiqul Islam, 2017. Effect of fish oil on performance, coccidiosis prevention and serum lipid profile in broiler. Asian J. Poult. Sci., 11: 20-30.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish oil contains n-3 polyunsaturated fatty acids (PUFA) especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Omega-3 PUFAs plays an important role in improving immunity, lipid profile and marketing weight of broilers[1,2]. Researchers in previous study stated that addition of fish oil in the diet of broilers results in an increased live weight of birds[3-5].

Chicken coccidiosis is a parasitic disease causing high morbidity and mortality in commercial poultry[6]. At present it is considered as one of the major problems for poultry industry throughout the world[7,8]. In Bangladesh coccidiosis is a major burden in chickens causing a great economic loss to the poultry farmers[9]. The causal agents of coccidiosis are several species of the genus *Eimeria* and *Isospora* belonging to the phylum Apicomplexa with a complex life cycle and affect mainly the intestinal tract of mammals and birds[10]. The degree of pathogenicity varies with causal agents and types of infection[11,12]. Typical symptoms of the disease (i.e., bloody faeces, diarrhea, reduced weight gain, depression, defeathering and increased mortality) are identified in affected birds in clinical coccidiosis. Subclinical coccidiosis is characterized by no visible symptoms of the disease[9,13]. Commercially reared broilers are vulnerable to coccidiosis leading to malnutrition, low growth performance and production efficiency of poultry[14]. Prophylactic use of anticoccidial drugs or vaccines promotes drug resistance to broilers[15]. Acquiring cross resistance to one drug by the use of another drug is also observed in broilers[16]. Feeding diets rich with n-3 (omega-3) fatty acids specially fish oil significantly reduces caecal lesions caused by coccidiosis. Oxidative stress induced by oxidation of the highly unsaturated fatty acids cause antiparasitic activity of the diet[14,17,18].

Fish oil also increases High Density Lipoprotein (HDL) cholesterol and decreases cholesterol, Low Density Lipoprotein (LDL) cholesterol, triglycerides level in blood of broilers[19-21]. Low HDL and high LDL in blood are the two important values associated with atherosclerosis and coronary heart disease in human. These values can be changed by change in regular diet[22,23]. It is revealed that dietary n-3 polyunsaturated fatty acids (PUFA) have well known effects on human health. In human, omega-3 (PUFAs) fatty acids are essential for prevention of coronary heart disease, hypertension, inflammatory, autoimmune disorders, cancer and several other diseases[24,25].

With this background, the present study was undertaken to observe the influences of fish oil on growth performance of commercial broilers to determine the effect of fish oil in preventing coccidiosis of broilers with gross, histopathological changes in affected birds and to test their serum lipid profile. The main objective was to establish regular use of Fish Oil (FO) in drinking water of broilers.

MATERIALS AND METHODS

The study methodology was designed into three sub-divisions to see the affectivity of supplementing fish oil on (1) Performance, (2) Preventing coccidiosis and (3) Serum lipid profile of broilers.

Location and duration of the experiments: The experiment was carried out from September, 2014 to February, 2015 at the Department of Animal Science and Nutrition Experimental Farm and Research Laboratories of Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, Bangladesh.

Ethical approval: After explanation of the objectives of the study consent was taken from ethical committee of Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University for using and slaughtering minimum number of birds.

Experimental design: Completely Randomized Design (CRD) was used in this study. One hundred and twenty birds were randomly distributed into four treatment groups after taking weight. Each treatment was divided into three replicates having 10 birds in each replicate. The dietary treatment groups were T_0 (control), T_1 (Basal diet+1% fish oil in drinking water), T_2 (Basal diet+2% fish oil in drinking water), T_3 (Basal diet+3% fish oil in drinking water), respectively.

Birds and housing: A total of 120 day-old chicks (Cobb 500 strain) of mixed sex were purchased from chicken hatchery. Before that the broiler shed, brooding boxes and broiler cages were thoroughly washed and cleaned by using tap water with caustic soda and phenyl solution. For killing microorganisms, phenyl solution (according to the manual) was also spread on the floor, corners and ceiling. After 1 week, lime was spread on the floor and around the shed for strictly maintaining bio-security. Footbath containing potassium permanganate was kept at the entrance of the poultry shed. Floor space for each bird was 0.0157 m^2 in brooding box and 0.53 m^2 in the cage. Room temperature and humidity was maintained using 200 W incandescent lamps and exhaust fans. Single-tired electric brooder was used for brooding chicks. The

temperatures maintained in 1st, 2nd, 3rd and 4th weeks were 308.15, 305.37, 302.59 and 299.82 K, respectively.

Collection of ingredients: Raw feed ingredients were collected from retail and wholesale market. Fish oil was collected from Bazarghata of Cox's Bazar, Bangladesh where marine fish oil is available. During collection it was assured that fish oil was fresh and free from dust or any other foreign particles. Essence of banana was collected from local market of Khatunganj, Chittagong, Bangladesh.

Diets: Birds were provided dry mash feed. The feeding standard was followed by Bangladesh standard specification for poultry feeds (2nd Revision, BDS 233: 2003; Bangladesh Standards and Testing Institution). The formulated diets were analyzed following instruction of AOAC[26]. All the rations were iso-energetic and iso-nitrogenous. Starter ration was supplied for 0-14 days of chicks and grower ration for 15-30 days (Table 1).

Fatty acid analysis of fish oil and mixing with water: At first fatty acid analysis of collected fish oil was done by gas chromatography according to the method described by previous researchers[27]. The composition of different fatty acids were recorded (Table 2). Fish oil was supplemented with regular drinking water from 1st day to the end of experiment. At first, little amount of fresh clean drinking water and fish oil was taken into a beaker. Essence of banana (6 mL L^{-1} of water) was also added to remove undesirable odour of fish oil. To dissolve fish oil with water, little amount (0.5 g L^{-1} of water) of emulsifier (Eurolipid®, ACI limited) was added. The mixture was stirred thoroughly in hot plate magnetic stirrer. After cooling, this mixture was added to large volume of regular drinking water of broilers.

Vaccination: All birds were vaccinated properly against Newcastle disease on the 4th days and booster dose again on 14th days.

Recording of data: Body weight of the chicks was recorded at 1st day and then weekly intervals. This measure was done along the whole experimental period. Weekly feed intake was calculated by deducting the left over feeds from the total amounts of supplied feed to the broilers. The body weight gain was calculated by deducting initial body weight from the final body weight of the birds. Feed Conversion (FC) of broilers was determined from the ratio between feed intake and body weight gain for weekly interval. Water intake by birds was also recorded throughout the whole experiment.

Table 1: Ingredients and nutritive composition of the experimental broiler diets

Ingredients (kg/100 kg)	Starter ration (0-14 days)		Grower ration (15-28 days)	
Maize	55.49		61.0	
Auto rice polish	4.5		0.4	
Full fat soya	3.0		1.5	
Molasses	0.8		0.5	
Vegetable oil	1.25		2.5	
Soybean meal	28.0		27.6	
Meat and bone meal	4.0		3.8	
Protein concentrate	0.5		0.5	
Limestone	1.0		1.0	
DCP	0.3		0.2	
Salt	0.3		0.3	
Vitamin mineral premix	0.30		0.25	
L-lysin	0.2		0.20	
DL-methionine	0.24		0.10	
Toxi-mold	0.03		0.05	
Enzyme	0.05		0.05	
Antioxidant	0.04		0.05	
Nutrient content (DM basis) (g/100 g)	Formula	Analysis	Formula	Analysis
Crude protein	21.85	22.03	21.00	21.02
Crude fiber	3.82	3.75	3.34	3.25
Crude fat	4.98	4.10	5.55	4.90
Calcium	1.07	0.97	1.01	0.95
Phosphorous	0.76	0.54	0.69	0.58
Lysin	1.42	1.29	1.36	1.20
DL-methionine	0.56	0.48	0.40	0.38
Metabolizable energy (MJ kg^{-1})	12.26	12.25	12.68	12.67

Table 2: Fatty acids composition of fish oils

Fatty acids	Identification	Retention times (min)	Fatty acids composition (% mol)
Myristic	C14:0	19.2	6.8
Palmitic	C16:0	21.3	18.9
Stearic	C18:0	24.6	3.6
Oleic	C18:1	25.9	20.4
Linolic	C18:2	27.4	6.8
Linolenic	C18:3	29.6	8.6
Eicosapentaenoic acid (EPA)	C20:5	35.2	14.9
Docosahexaenoic acid (DHA)	C22:6	38.8	15.7

Determining coccidiosis in broilers: In earlier experiments it has been reported that infection with 10^4 oocysts of *Eimeria* produce a moderate to severe infection with a very low rate of mortality[9,28]. Therefore, 10^4 sporulated oocysts were used as infective dose in this experiment.

Production of fresh oocysts for experimental infection: Seven-day old chicks were infected with 10^4 sporulated oocysts of *Eimeria* (collected from for Pathology and Parasitology Laboratory, CVASU) for production of fresh oocysts. Oocysts from faeces were collected from 6 days post infection (dpi). Cleaning of oocysts was done by adopting the method suggested by Ryley *et al.*[29].

Preparation of oocysts dose: Firstly, oocysts suspension was centrifuged at 500×g for 5 min in test tubes. After that the supernatant was discarded. The sediment was then suspended in distilled water. Centrifugation was repeated and the final sediment was resuspended again in distilled water. By using McMaster counting technique the number of oocysts per milliliter was counted following the methos described by Soulsby[30]. Finally the number of oocysts as infective inocula (10^4 sporulated oocysts) was adjusted to a volume of 0.5-1 mL with water.

Infection with oocysts: All birds from T_0 (without FO), T_1 (1% FO), T_2 (2% FO) and T_3 (3% FO) groups were infected with 10^4 sporulated oocysts for each bird. The infective inocula were introduced directly into the crop of the chick using a plastic dosing tube attached to a 1 mL plastic syringe.

Recording of clinical sign: Clinical signs (i.e., bloody faeces with diarrhea, loss of weight, flecks of bloods and mucous in faeces, sleeping tendency, depression and weakness of affected birds) were recorded from the day of infection till the end of the experiment. Mortality was recorded throughout the experimental period when death occurred in any replication.

Necropsy and gross lesion: At 8 days post infection (dpi) six birds from each dietary treatment groups were sacrificed. The whole intestine including the caeca was removed from the sacrificed and dead birds. Following previous study, gross lesions for various species were noted and scored ranging from a scale of 0 (no gross lesion) to 4 (most severe gross lesion) following the method of previous researchers[31].

Histopathological examination: The intestinal and caecal samples which were fixed in 10% neutral buffer formalin examined for histopathology as described by Bancroft and Stevens[32].

Serum lipid profile: Blood was collected without using anticoagulant from 6 birds of each group at 30th days of age of broilers. Serum was separated after centrifugation at 3,000 rpm for 15 min. Serum cholesterol, HDL-cholesterol, LDL-cholesterol levels were measured using standard kits (BioMereux, France) and automatic analyzer (Humalyzer 300, Merck®, Germany) according to the manufacturer's instruction (FVMAAU, Addis Ababa, Ethiopia).

Statistical analysis: All the data related were entered into MS excel (Microsoft office excel-2007, USA). Data management and data analysis were done for ANOVA by STATA version-12.1 (STATA Corporation, College Station, Texas). A p-values of either $\leq$0.05 or $\leq$0.01 were considered statistically significant and highly significant, respectively.

RESULTS AND DISCUSSION

Effect of fish oil on performance of broilers

Feed consumption: Table 3 represents the amount of feed consumption of birds in different ages of birds. At 1st week of age the values of different groups were non significant ($p>0.05$). Though the feed consumption was increased at 2nd weeks of age in birds which were supplied with 1% fish oil, the values among different dietary treatment groups did not differ significantly ($p>0.05$). Both at 3rd and 4th weeks of age, there was a highly significant ($p<0.01$) increase in daily feed consumption among the groups treated without fish oil.

Live weight: Table 4 shows that no significant difference ($p>0.05$) was found initially in live weight among the birds of different dietary treatment groups. The differences in live weight of broilers at 1st and 2nd weeks of age were also not significant ($p>0.05$) among different groups. But at 3rd weeks of age highly significant ($p>0.01$) difference was found in different treatment groups. At 4th weeks of age difference in live weight among broilers was also highly significant ($p<0.01$) and was higher in 2 and 3% fish oil dietary treatment groups in comparison with other two groups.

Live weight gain: Live weight gain of broilers in different dietary treatment groups of broilers was also non significant ($p>0.05$) statistically both at 1st and 2nd weeks of age (Table 5). Highly significant ($p<0.01$) difference in weight gain of broilers in different treatment groups were observed in 3rd and 4th weeks of age which was higher in 2 and 3% fish oil treatment groups.

Feed conversion: Feed conversion of broilers among different treatment groups differed non significantly ($p<0.05$) upto 2nd weeks of age of broilers though it was higher in control group in comparison with other groups. Significantly ($p<0.01$) higher feed conversion was found at 3rd and 4th weeks of age in control and 1% fish oil dietary treatment groups compared to 2 and 3% fish oil supplemented groups with highly significant ($p<0.05$) difference (Table 6).

Water consumption: Water consumption by T_0, T_1 and T_2 and T_3 groups were 597, 660, 876, 896 mL bird^{-1},

Table 3: Weekly feed consumption of broilers among different dietary treatment groups (g broiler^{-1})

Age	T_0 (Control)		T_1 (1% FO)		T_2 (2% FO)		T_3 (3% FO)			
(Weeks)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value	Level of significance
1	158.21	20.26	165.9	11.7	167.0	9.95	164.4	10.2	0.064	NS
2	343.63	24.58	346.5	43.5	336.2	11.8	336.9	13.6	0.357	NS
3	640.65	69.92	618.5	30.0	708.4	34.8	715.9	21.3	<0.0001	**
4	586.40	128.20	591.4	66.6	742.9	47.8	725.8	36.9	<0.0001	**

FO: Fish oil, SD: Standard deviation, NS: Non significant, **Significant at 1% level

Table 4: Weekly body weight of broilers among different dietary treatment groups (g broiler^{-1})

Age	T_0 (control)		T_1 (1% FO)		T_2 (2% FO)		T_3 (3% FO)			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value	Level of significance
Day 1	34.85	4.44	32.91	3.36	33.116	3.99	34.15	3.63	0.163	NS
1st week	138.66	12.50	140.76	5.66	142.79	4.98	141.62	5.29	0.2186	NS
2nd week	341.14	7.96	347.44	9.14	348.15	2.60	345.40	1.57	0.580	NS
3rd week	727.57	12.10	734.59	15.1	808.967	18.65	814.73	10.30	<0.0001	**
4th week	964.95	42.90	1005.90	26.6	1281.96	26.86	1295.40	22.03	<0.0001	**

SD: Standard deviation, NS: Non significant, **Significant at 1% level

Table 5: Weekly body weight gain of broilers among different dietary treatment groups (g broiler^{-1})

Age	T_0 (Control)		T_1 (1% FO)		T_2 (2% FO)		T_3 (3% FO)			
(Weeks)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value	Level of significance
1	103.8	13.3	107.8	7.94	109.6	6.19	107.4	5.90	0.08	NS
2	202.6	14.9	206.6	11.9	205.3	6.07	203.9	5.60	0.84	NS
3	386.4	15.8	387.1	16.3	460.8	19.7	469.3	10.10	<0.0001	**
4	237.3	50.8	271.4	33.1	472.9	28.3	480.6	24.40	<0.0001	**

SD: Standard deviation, NS: Non significant, **Significant at 1% level

Table 6: Weekly body feed conversion (FC) of broilers among different dietary treatment groups (g broiler^{-1})

Age	T_0 (Control)		T_1 (1% FO)		T_2 (2% FO)		T_3 (3% FO)			
(Weeks)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value	Level of significance
1	1.524	0.02	1.539	0.03	1.523	0.01	1.530	0.056	0.31	NS
2	1.695	0.02	1.676	0.05	1.637	0.03	1.65	0.044	0.09	NS
3	1.66	0.13	1.590	0.02	1.538	0.05	1.525	0.044	<0.0001	**
4	2.470	0.04	2.180	0.14	1.57	0.03	1.51	0.049	<0.0001	**

SD: Standard deviation, NS: Non significant, **Significant at 1% level

respectively throughout the whole experiment. This difference was significant ($p<0.05$), statistically.

Effect of fish oil on coccidiosis prevention in broilers

Clinical signs: In this experiment birds from T_0 (without fish oil) and T_1 (1% fish oil) groups developed signs of coccidiosis including bloody diarrhoea and weight loss (infected). No visible signs of coccidiosis were observed in birds treated with 2 and 3% fish oil (uninfected).

Gross lesions: All the birds sacrificed from T_0 (without fish oil) group including two dead birds revealed highly swollen caeca with full of clotted blood and blood mixed feces. Large numbers of haemorrhagic and necrotic spots on the mucosal surface with oocysts in mucosal scrapings were also observed. There were mottled reddish or milky white colored contents in the caeca due to formation of oocysts. Those birds were scored either 3 or 4. Birds which received 1% fish oil also showed gross lesions of coccidiosis such as hemorrhage, bloody faeces with a hyperemic, swollen and thickened caecal wall. But those lesions were not so severe like control group. So they were scored either +1 or +2 (Fig. 1b, c). No gross lesions of coccidiosis were observed in the intestines of birds receiving 2 and 3% fish oil (Fig. 1d, e). They were scored as 0 (Fig. 1a).

Histopathological lesions: Histopathological examination of the affected caeca and other intestinal parts demonstrated characteristic microscopic signs of coccidiosis in the affected broilers (Fig. 2). Caecal sections from control group revealed second generation schizont which was a clear indication of coccidiosis (Fig. 2a). Excessive tissue damage with

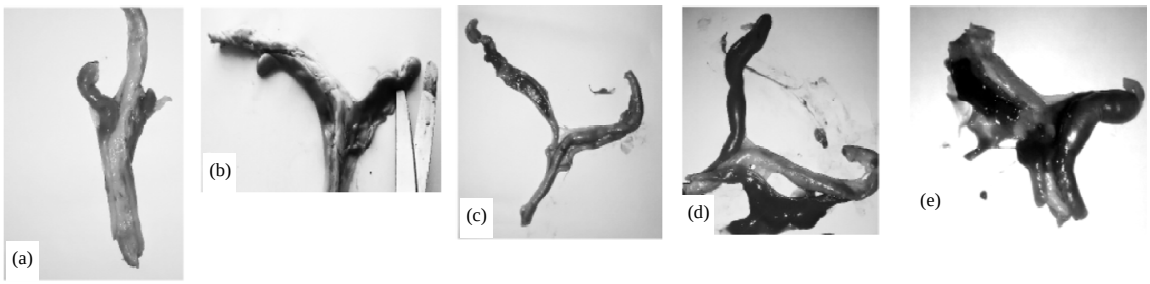

Fig. 1(a-e): Gross lesions in intestinal sections of birds affected with coccidiosis, (a) Intestinal part collected from bird treated with 2% fish oil showing no visible gross lesions of coccidiosis (scored as "0"), (b, c) Slight hemorrhage and blood mixed faeces in caecal sections taken from birds treated with 1% fish oil (scored as "1" and "2", respectively) and (d, e) Enlarged and distended caeca with more bloody faeces in birds of control (scored as "3" and "4", respectively)

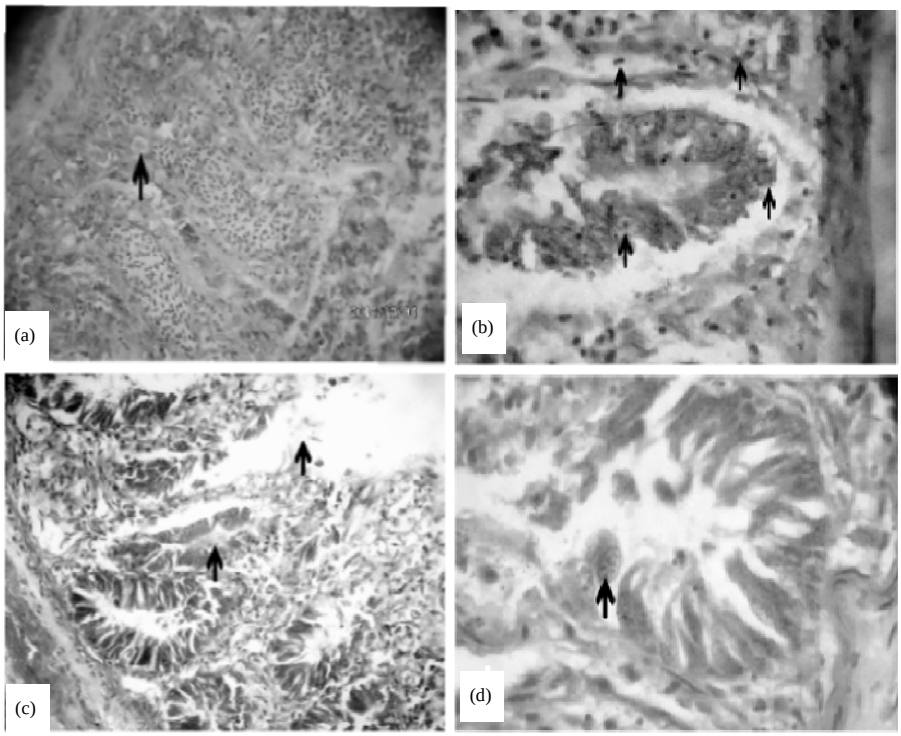

Fig. 2(a-d): Histopathological changes in intestimal sections of coccidiosis affcted birds, (a) Second generation schizont of *Eimeria tennella* in caecal section taken from bird of control group (40X), (b) Developmental stages (sporozoite, merozoite, macrogametocyte and microgametocyte) of *Eimeria* in intestinal sections of control group (40X), (c) Haemorrhage, destruction of intestinal epithelium of birds treated with 1% fish oil (40X) and (d) Microgametocyte of *Eimerc* in caecal section of 1% fish oil treatment group (100X)

haemorrhage was also present in those sections. Different deveplopmental stages like sporozoites, merozoites, microgametocytes, macrogametocytes of *Eimeria* were also observed in tissue of intestinal sections from the same group (Fig. 2b). Intestinal sections from 1% fish oil treatment group demonstrated severe tissue damage in the epithelium (Fig. 2c). Microgametocytes of *Eimeria* with huge number of matured oocysts revealed in caecal sections (Fig. 2d). However, no histopathological changes were observed in intestinal sections taken from birds of T_2 (2% FO) and T_3 (3% FO) groups (uninfected).

Effect of fish oil on serum lipid profile of broilers: It was noted that serum HDL was increased and total serum cholesterol, LDL, triglycerides was reduced significantly ($p<0.01$) in T_2 (2% fish oil) and T_3 (3% fish oil) groups in comparison with T_1 (1% fish oil) and control groups at 30 days of age of broilers (Table 7).

Table 7: Serum lipid profiles of broilers at 30th days of age fed on diets supplemented with different percentage of fish oil or without oil (mg dL^{-1} $broiler^{-1}$)

	T_0 (Control)		T_1 (1% FO)		T_2 (2% FO)		T_3 (3% FO)			
Parameters	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p-value	Level of significance
Serum cholesterol	155.80	1.86	115.66	0.8	106.60	1.3	101.78	0.9	<0.0001	**
HDL-cholesterol	15.60	0.70	17.33	0.5	20.01	0.4	22.03	0.7	<0.0001	**
LDL-cholesterol	72.93	2.84	70.73	1.6	60.49	3.3	65.66	3.3	0.0006	**
Triglycerides	157.20	1.04	117.30	0.8	89.93	2.5	97.11	1.0	<0.0001	**

SD: Standard deviation, **Significant at 1% level

Performance of broilers: In this study no significant difference ($p>0.05$) was found in feed consumption of birds supplemented with or without fish oil at 1st and 2nd weeks of age. However, highly significant difference ($p<0.01$) was observed at 3rd and 4th weeks of age where birds supplemented with 2 and 3% fish oil to drinking water showed increased consumption of feed (Table 3). Saleh *et al.*[20] reported that inclusion of 1.5% of fish oil in poultry diet increases feed intake which is in agreement with the present findings. In tandem with this, supplementation of 3% fish oil resulted higher ($p<0.01$) feed intake than those on other oils or without oils[5]. Increased food consumption was observed compared to the control group in a study even in higher (8.2%) percentage of fish oil[4]. Chekani-Azar *et al.*[33] reported that fish oil containing diets decrease feed intake of broilers. The cause of lower feed intake by broilers can be due to higher digestibility of the fat component of PUFA rich diet involving a higher dietary content of metabolizable energy and thus less feed needed to meet the energy requirement.

Table 4 indicates that initial body weight of birds among different groups differed slightly but those values were not significant statistically ($p>0.05$). It indicates a higher possibility of having similar weighted birds in different groups prior the beginning of the treatment. The non significant ($p>0.05$) results were also observed both at 1st and 2nd weeks of age. Live weight of broilers was improved significantly ($p<0.01$) in FO treatments compared to control group at the end of the experiment (3rd and 4th weeks). At 1st and 2nd weeks of age, weight gain in T_0 (Control) and T_1 (1% fish oil) dietary treatment groups was observed less compared to T_2 and T_3 groups (2 and 3% fish oil treated groups, respectively). However, those differences were not significant ($p>0.05$) statistically. Effect of fish oil was more pronounced with the advancement of age. Inclusion of fish oil to diet improved body weight gain of broilers significantly ($p<0.01$) at 3rd and 4th weeks of age. Highest live weight gain was found in broilers supplemented with 2 % fish oil and lowest live weight gain was in broilers of control group at 4th weeks of age (Table 5). The results of increasing live weight gain with the addition of fish oil in the diet of broilers are in agreement with many previous findings[3,34]. Previous studies revealed that, omega-3 PUFAs improve lipid profile and marketing weight of broilers[1]. Significantly ($p<0.05$) higher live weight gain was reported with the addition of fish oil compared to control and other oil treated groups[5].

Supplementation of fish oil at different percentages with regular drinking water of broilers resulted a significant impact in feed conversion with the advancement of age though it was insignificant ($p>0.05$) up to 2nd weeks of age. At 3rd weeks of age feed conversion was significantly ($p<0.05$) better in the birds receiving 2 and 3% fish oil with regular drinking water. However, best FC was found in broilers supplemented with 3% fish oil at 4th weeks of age which differed significantly ($p<0.05$) from other groups. Next to this group 2% fish oil supplemented groups showed improved ($p<0.05$) FC (Table 6). Similar findings of better feed conversion with supplementing fish oil at different levels in diet of broilers were recorded previously by several researchers[33]. The findings of the present study are in disagreements with some researchers[25,35]. They recorded that daily feed intake and feed conversion was not affected by dietary fish oil. Good performance of fish oil fed broilers may be related to the fatty acid composition of fish oil[5].

Water consumption by birds among different treatment groups differed significantly ($p<0.05$). Water intake increased significantly with increasing level of fish oil which is concordant with previous findings[36]. This difference may be due to composition of fatty acids in water.

Coccidiosis prevention in broilers: Several researchers in previous studies recorded that omega-3 PUFAs plays an important role in improving immunity of broilers[1,34]. Control of Coccidiosis is possible by inclusion of omega-3 fatty acids in the diet. Researchers have reported that fish oil is a valuable ingredient to enrich poultry meat because of higher amount of EPA and DHA[25,37]. It is possible to infiltrate tissues of the parasite by highly unsaturated omega-3 fatty acids. After infiltration they become more susceptible to oxidative attack

by phagocytic cells. Subsequently development of coccidian is adversely affected by this oxidative stress[38,39].

In this experiment clinical coccidiosis was seen with clotted and unclotted blood in faeces among the birds in which no dietary fish oil was used (T_0) at 4 days post infection (dpi). Lesions were aggravated on 6 and 7 dpi. At 7 dpi almost all the birds from control groups were infected. Fresh bloody discharge was found in the droppings of affected birds. Depression, weakness, bloody diarrhoea, anorexia and ruffled feathers were also observed. Birds which received 1% dietary fish oil also showed same clinical signs but the lesions were not so severe like control group (T_1). On 5 and 7 dpi two coccidian infected birds from control group were died. The signs of coccidiosis were similar as those mentioned by several authors[6,13]. However, birds which were fed 2 and 3% fish oil (T_2 and T_3 groups) with regular drinking water showed very minimal or no clinical signs.

At necropsy all the birds from T_0 (Control) and T_1 (1% fish oil) groups revealed gross lesions of coccidiosis which were characterized by enlarged and distended caeca filled with blood and petechial hemorrhages in some parts of the lower intestine (Fig. 1). The severity of infection was more in control group than 1% fish oil treatment group. Similar gross lesions of coccidiosis were also recorded previously by some authors[6,9,13]. According to previous study, all the birds from control group developed severe lesions and scored[31] from +3 to +4. Due to reduction of lesion birds from 1% fish oil treated group were scored between +1 to +2. However, birds from T_2 (2% fish oil) and T_3 (3% fish oil) groups remains uninfected and developed no lesions of coccidiosis. They were scored 0 in lesion scoring method. Researchers in previous studies recorded that lower level of fish oil (1%) reduced lesion score of coccidiosis non significantly ($p>0.05$)[18,40]. Allen and Danforth[18] reported a significant ($p<0.05$) reduction of gross lesions resulted from coccidiosis with the addition of 5% fish oil to basal diet of broilers and no further improvement was observed in lesion score when fish oil was increased to 10%. Some researchers compared performance of broilers with the addition of both 4% corn and fish oil. They observed that fish oil reduced lesion scores though the result was non significant ($p>0.05$)[41].

Histopathology of intestinal sections and intestinal parts (specially caecum) taken from birds of infected groups (control and 1% fish oil) demonstrated characteristic microscopic lesions of coccidiosis (Fig. 2). Caecal sections from control groups revealed severe hemorrhage, necrosis of mucosa and submucosa, presence of clusters of oocysts. Second generation schizont (pathognomonic for *Eimeria tenella*) was also observed within the caecal cells (Fig. 2a). The result was in concordance with several researchers[13,42]. Microgametocytes, macrogametocyes, sporozoites and merozoites were also observed in the sections taken from epithelial cells of intestine of control group (Fig. 2b). Intestinal layers of birds treated with 1% FO revealed severe epithelial cell destruction in histopathology (Fig. 2c). There was disruption of epithelium followed by leakage of blood. Similar findings in intestinal sections of coccidian affected birds were also recorded by several researchers[13,43,44].

Serum lipid profile in broilers using fish oil: Table 7 interprets that there was a significant variation in mean values of total serum cholesterol level of birds ($p<0.05$) among different treatment groups. Highest value of total serum cholesterol level was found in bird of control (without fish oil) group and second highest value was found in birds treated with 1% fish oil. With increasing percentage of fish oil, level of total serum cholesterol was gradually reduced. Both 2 and 3% fish oil supplemented groups showed lower level of total serum cholesterol compared to control group and these differences were significant statistically ($p<0.01$). All these results indicate that with the addition of fish oil to broiler diet, amount of cholesterol is decreased. These results are in agreement with the findings of other studies[21,45]. However, a few studies observed that the level of serum cholesterol was not affected noticeably by dietary fish oil[3]. This difference may be attributed to the genetic, sex and dietary factors[20].

Supplementation of oils caused a highly significant ($p<0.01$) impact on serum HDL-cholesterol level of broilers (Table 7). Higher values of HDL-cholesterol were found in dietary fish oil supplemented groups compared to control group. Three percent fish oil supplemented group showed highest HDL-cholesterol compared to others. The second largest values were seen in birds with 2% fish oil supplemented group. Among these groups, lowest value ($p<0.01$) was observed in control group. The trend of increased HDL-cholesterol level of broilers with soybean and fish oil groups compared to palm oil was recorded by others researchers[21]. Inclusion of oil in low energy diet also caused an increase HDL level in serum of broilers[46]. Researchers reported that low HDL are values associated with atheroschlorosis and coronary heart disease in human[4,22]. Regular diet has an effect on the levels of HDL in blood. It was claimed that in a healthy body the level of HDL is high[3].

Serum LDL-cholesterol level of blood varied significantly among the different treatment groups (Table 7). The LDL-cholesterol level was significantly ($p<0.01$) higher among the broilers in which no fish oil was supplemented. However, there was a decrease in LDL-cholesterol level with increasing

percentage of fish oil. One percent fish oil dietary supplemented groups showed highest value among the fish oil supplemented groups. Two percent fish oil supplemented group showed slightly highest value compared to 3% fish oil supplemented group. Das *et al.*[21] observed lower levels of LDL in serum of broiler groups supplemented with 3% fish oil but the values were insignificant ($p>0.05$)[19]. It was reported that omega-3 fatty acids reduce the blood very Low Density Lipoprotein (VLDL) levels, acting to lower the circulating free LDL concentration[4,22].

Data in the Table 7 shows that supplementation of oil resulted a great effect on triglyceride level of blood. Significantly ($p<0.01$) higher level of triglyceride content was seen in serum of broiler group without dietary fish oil (control). Lowest value of triglyceride level was recorded in serum of 2% fish oil supplemented groups at 30th days of age. Several studies are in agreement with the present findings of decreased triglyceride level in the blood of broilers supplemented with fish oil[4,20]. Lower level of triglyceride with 2 and 4% fish oil compared to control group was recorded in previous study though the values were not statistically significant ($p>0.05$)[3].

CONCLUSION

Fish oil as a source of omega-3 fatty acids can be used in drinking water of broilers to increase their growth performance and productivity. Serum lipid profile parameters of birds also become better by using this oil. Use of anticoccidials in prevention and control of chicken coccidiosis causes resistance to drugs in broilers eventually in humans which is a burning issue of modern time. Supplementation of fish oil as a natural source may be a useful alternative in this regard. Two to three percent fish oil is recommended which contribute effectively both in preventing disease and improving performance. It will reduce health hazards of broilers and human. However, further study may be recommended to make a concrete result of the findings.

SIGNIFICANCE STATEMENT

This study mainly focuses on establishment of using fish oil in regular drinking water of broilers to increase growth performance, improving lipid profiles, productivity of broilers. It also reports on affectivity of fish oil as a natural source in preventing coccidiosis of broilers which is a major threat in poultry industry. Several types of antibiotics and coccidiostats are used in broilers feed to prevent coccidiosis and other diseases. Besides preventing coccidiosis, fish oil could be also a useful alternative to drugs in suppressing many other diseases by increasing immunity of broilers. This type of research of using fish oil in regular drinking water of broilers is few which might be a new era in poultry research.

ACKNOWLEDGMENT

We thank the University Grants Commission (UGC), Bangladesh for financial support.

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