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Effect of purified fish oil supplementation on growth performance, meat quality and blood profile in broilers

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Abstract

The present research experimented with the view of observing the effect of supplementing purified fish oil (FO) on growth performance, organ weight, meat quality, along with blood profile of broilers. The experiment was conducted upon 1600 male Ross 308, each aged 1 day, broiler strains with an average body weight of 43.15 ± 1.35 g were randomly allotted into four dietary treatment groups where CON, basal diet; TRT1, CON + 0.05% FO; TRT2, CON + 0.10% FO; and TRT3, CON + 0.15% FO respectively. Each treatment group consisted of 20 replication pens, with each pen containing 20 broiler chicks. The 4-week long experiment was divided into two phases. The first phase (Days 7–21), the second phase (Days 22–35) and the overall (Days 7–35). On Day 21, the body weight of broilers in TRT2 and TRT3 was higher than the control. Increasing doses of purified FO supplementation showed a linear ($p < 0.05$) increase in body weight on Day 21. At the end of Phase 1, FO supplementation improved the body weight gain (BWG) of TRT2 and TRT3 group birds compared to CON. Increasing doses of FO supplementation increased the BWG linearly in all phases. Increasing doses of FO increased chicks' breast muscle weight with the highest value in the TRT3 group. Moreover, liver weight was linearly decreased in FO supplementation groups compared to the CON group. Abdominal fat was linearly decreased in FO supplemented groups. FO supplementation caused the lowest cholesterol and triglyceride in TRT3 than the CON group with a linear reduction. Low density lipoprotein (LDL) cholesterol was reduced by FO supplemental groups. In addition, FO supplementation increased the red blood cell (RBC) in the TRT3 group compared to the CON group. In conclusion, refined FO increased BWG, breast muscle weight and RBC and reduced cholesterol, triglycerides and LDL in broilers' blood.

KEYWORDS

blood profile, broiler, fatty acid, fish oil, performance

1 | INTRODUCTION

There is a competition for fat sources between the human and animal food industries, as natural oil sources like crops, herbs, marine and animal origins are common resources in both markets. According to Leeson and Summers (2005), fats and oils are considered an outstanding source of energy in the diet since they are rich in n-3 polyunsaturated fatty acids (N-3 PUFA). There is much controversy regarding the relative merits of monounsaturated, n-6-polyunsaturated, and n-3-PUFA in the human diet, as research has focused on the roles of linoleic acid (C18:2n-6) and linolenic acid (C18:3n-3) as precursors to arachidonic acid (C20:4n-6), eicosapentaenoic acid (EPA) (C20:5n-3) and the eicosanoids derived

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therefrom, and the role of the C20 and C22 PUFA in marine oils (Scaife et al., 1994). Animals are unable to synthesise linoleic acid (LA) and alpha-linolenic acid (ALA) as the metabolism and the role of PUFA n-6 and n-3 differ, which leads Woods and Fearon (2009) to suggest that PUFA should be consumed through diet as dietary changes are capable of stimulating rapid responses in tissue concentration (Wood & Enser, 1997). With a view to modifying their diet with enriched PUFA, monogastric animals are more susceptible to absorbing and depositing dietary fatty acids in tissue. Bhalerao et al. (2014) suggested that, in order to increase the amount of desired PUFA present in chicken meat, changes in the composition of dietary fatty acids are one of the best approaches, which is advantageous for increasing birds' health performance as well. The addition of ALA and fish oil (FO) proved to improve the lipid composition of broilers meat (Lopez-Ferrer et al., 1999; Scaife et al., 1994). The high content of omega-6 fatty acids in FO may prevent the desaturation of n-6 fats, causing a decrease in lipid levels in the blood (Z. B. Huang et al., 1990). Several researchers, Chanmugam et al. (1992), Miller and Robisch (1969), Pinchasov and Nir (1992) have demonstrated that feeding fish meal to hens makes it possible to increase the level of carbon-based omega-3 fatty acid in broiler muscle. In addition, small amounts of fat and oil supplementation in broiler diets have been demonstrated to stimulate growth and feed efficiency while improving the consistency and palatability of the meat.

A higher degree of unsaturation, however, will also lead to more oxidation susceptibility in poultry meat with a higher PUFA content, which has the possibility to create off-odours and off-flavours. Pinchasov and Nir (1992) noticed the increment in α-linoleic acid in broiler fat with an increasing PUFA supplementation using a composition of different vegetable oils and beef fat. Hulan et al. (1988); Chanmugam et al. (1992) investigated the increment in C20 and C22 n-3 PUFA concentrations of poultry tissue using marine oils and fish meal containing residual lipid. Hulan et al. (1988) included FO in the diet provided for broilers and observed substantial deposition of EPA, docosahexaenoic acid (DHA), and a few other n-3 PUFA in the carcass and the edible meat lipids. An analysis of n-3 PUFA and lipid contents of meat revealed an important influence of FO Ajuyah et al. (1992) and H. C. Huang, 1996).

An experiment about the effect of FO supplementation on humoral immune response and some blood biochemical parameters of broiler chickens was conducted by Mansoub and Yadollah Bahrami (2011), where they noticed that the addition of FO in the broiler diet might be responsible for stimulating the development of the immune response as well as improving blood indices. Similarly, Maroufyan et al. (2013) suggested that FO supplementation acquired higher levels of total protein, white blood cell (WBC) count and IL-2 concentration in broilers infected with bursal disease challenges. It is believed that omega-3 PUFAs can help birds to develop their immune system strong enough to resist infection, increase poultry production and reduce disease, thus contributing to the growth of the poultry industry. Inconsistent results in growth performance (Guo et al., 2020; Øverland et al., 1996; Komprda et al., 2020), blood lipid profile (Almeida et al., 2021; Komprda et al., 2020) and adverse effects in sensory meat (S. A. Lee et al., 2019; Ribeiro et al., 2013). The inconsistency was due to the different doses and purity of FO. So, our study was evaluating a lower dose of purified FO on growth performance, blood lipid profile and meat quality of broiler.

Therefore, we hypothesised that a purified FO supplementation will help with growth performance, desirable meat quality, healthy lipid profile and better immune status in broilers.

The objective of this study was to determine the effect of purified FO supplementation in growth performance, organ weight, meat quality and blood lipid profile of broilers.

2 | MATERIALS AND METHODS

This study protocol (DK-1-2014) and animal ethics guidelines were approved by the Animal Care and Use Committee of Dankook University, South Korea, and the present study was conducted in compliance with them. The purified FO tested in this experiment was supplied by Morningbio. As per the supplier's information, the tested product was produced through the purification of FO through trans esterification and molecular distillation process according to the method described by Hoque et al. (2011). The purified product was powdered and absorbed on a support comprising silica. The fatty acid composition of purified FO includes myristic acid (4%), palmitic acid (49%), palmitoleic acid (3.8%), stearic acid (27%), oleic acid (3.5%), linoleic acid (0.5%), α-linoleic acid (0.5%), EPA (4.5%) and DHA (1.3%) (Table 1).

2.1 | Animals and housing

Chickens were brought into an experimental unit of Dankook University, Korea that had been cleaned and disinfected before arrival. On the same day, the broilers were administered the vaccine and they were weighed to establish their initial body weight. Thousand six hundred male Ross 308 broiler chicks with an initial body weight averaging 43.15 ± 1.35 g were used in 28 days trial. In this experiment, the initial temperature was set to 33 ± 1°C for the first week, gradually decreasing to 24 ± 1°C for the following weeks. Throughout the experiment, 60% humidity was

TABLE 1 Fatty acid composition of the tested product

Fatty acid	Carbon molecules	Percentage (%)
Myristic acid	C 14	4
Palmitic acid	C 16	49
Palmitoleic acid	C 16:1	3.8
Stearic acid	C 18	27
Oleic acid	C 18:1	3.5
Linoleic acid	C 18:2	0.4
α-Linolenic acid	C 18:3	0.5
EPA	C 20:5	4.5
DHA	C 22:6	1.3

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

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maintained inside the experimental facility. Every pen size was (1 m × 1.6 m) and stocking density was less than 25 kg/m^2. Every pen was equipped with a nipple and feeder that allowed birds to freely access nutritious food and clean water for the duration of the experiment. Eighteen hours of LED lighting was provided per day with a 6-h dark period. The broiler room was routinely cleaned for hygiene purposes during the experiment.

2.2 | Experimental diet and fatty acid composition

A total of 1600 broilers were randomly allocated into four treatment groups for the purpose of the research, with 20 replications per treatment consisting of 20 birds per pen. For the first week, the broilers were provided with a basal diet. Supplementation with FO into the diet started from Day 7 and continued to Day 35. The dietary treatments involved: CON, basal diet; TRT1, CON + 0.05% FO; TRT2, CON + 0.10% FO; and TRT3, CON + 0.15% FO. As per the suggestions of Rostagno et al. (2011) the basal diet was formulated to maintain the nutritional requirements (Tables 2–4). To extract fat from the samples, three diet samples were macerated in hexane solvent (5:1) at room temperature for 24 h. From the filtrated samples, a solvent containing soluble fat was obtained, which was then kept at 35°C to remove the hexane. Fat samples were placed in Falcon tubes and maintained in the refrigerator to keep them fresh. Saturated, unsaturated and total fatty acid levels were assessed by measuring methyl esters and boron trifluoride content in oil (AOAC, 2000). The oil was saponified by mixing sodium hydroxide and methanol. A combination of 12 fatty acids was methylated with boron trifluoride and extracted with heptane, then analysed using carrier gas (He) (approximately 25 psi—air 450 ml—split 100 ml/min) on a gas chromatograph with a flame ionization detector system, auto-sampler and EZChrom integration system. The oven and injector were both heated to 200°C throughout the test, while the detector was heated to 250°C. The analysed fatty acid compositions of the experimental diets are presented in Table 5. Purified FO supplementation decreased palmitic acid, stearic acid and linoleic acid in PFO-added diets more than in control. As FO contains a high amount of DHA so it increased the DHA concentration in the experimental diets more than control.

2.3 | Measuring body weight and feed intake (FI)

Body weight gain (BWG), FI and feed conversion ratio (FCR) were determined by weighing all 1600 broilers by pen on Days 7, 21 and 35. These measurements determine the growth performance of the broilers. Dividing FI with BWG resulted in achieving a FCR.

2.4 | Examining blood profile

At the very end of the experiment, blood samples from 20 broilers (1 bird/pen) per treatment were collected from the wing vein located over the medial surface of the proximal ulna. After collecting, the blood samples were stored in clean vacuum tubes having no additive to obtain the serum, and to obtain the whole blood, tubes containing K3EDTA (Becton Dickinson Vacutainer Systems) were used. We measured the total concentration of blood cells like red blood cells (RBC), lymphocytes and WBC using an automatic blood analyzer (ADVIA 120; Bayer) and, in order to separate serum samples, blood samples were centrifuged for 30 min at 3300 rpm. We then analysed the serum using an automatic biochemistry analyzer (Hitachi 747; Hitachi) to find out its lipid profile.

TABLE 2 Feed composition of broiler (as fed-basis)

Item	Starter (0–7 days)			
	CON	TRT1	TRT2	TRT3
Ingredients (%)				
Corn	44.60	44.58	44.56	44.52
Soybean bran	34.13	34.11	34.11	34.11
Corn gluten meal	13.00	13.00	13.00	13.00
Wheat bran	3.00	3.00	3.00	3.00
Soyoil	1.76	1.75	1.72	1.70
Mono calcium phosphate	1.40	1.40	1.40	1.40
Limestone	1.37	1.37	1.37	1.38
Salt	0.36	0.36	0.36	0.36
Methionine (99%)	0.15	0.15	0.15	0.15
Lysine	0.03	0.03	0.03	0.03
Mineral mix[a]	0.10	0.10	0.10	0.10
Vitamin mix[b]	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00
Calculated value				
Crude protein (%)	23.00	23.00	23.00	23.00
Ca (%)	1.00	1.00	1.00	1.00
P (%)	0.74	0.74	0.74	0.74
Available P (%)	0.45	0.45	0.45	0.45
Lys (%)	1.26	1.26	1.26	1.26
Met (%)	0.50	0.50	0.50	0.50
Merabolizable energy (kcal/kg)	3200	3200	3200	3200
FAT (%)	4.46	4.48	4.49	4.51
Fibre (%)	3.55	3.55	3.54	3.54
Ash (%)	6.78	6.78	6.78	6.78

[a]Provided per kg of complete diet: 37.5 mg Zn (as $ZnSO_4$); 37.5 mg Mn (as MnO_2); 37.5 mg Fe (as $FeSO_4 \cdot 7H_2O$); 3.75 mg Cu (as $CuSO_4 \cdot 5H_2O$); 0.83 mg I (as KI); and 0.23 mg Se (as $Na_2SeO_3 \cdot 5H_2O$).

[b]Provided per kg of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 IU of vitamin E, 2.55 mg of vitamin K_3, 3 mg of Thiamin, 7.5 mg of Rivoflavin, 4.5 mg of vitamin B_6, 24 ug of vitamin B_{12}, 51 mg of Niacin, 1.5 mg of Folic acid, 0.2 mg of Biotin and 13.5 mg of Ca-Pantothenate.

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TABLE 3 Feed composition of broiler (as fed-basis)

Item	Grower (7–21 days)			
	CON	TRT1	TRT2	TRT3
Ingredients (%)				
Corn	47.70	47.75	47.61	47.59
Soybean bran	31.33	31.30	31.32	31.41
Corn gluten meal	13.05	13.00	13.00	13.00
Wheat bran	3.00	3.00	3.00	3.00
Soyoil	1.62	1.60	1.57	1.55
Mono calcium phosphate	1.20	1.20	1.20	1.20
Limestone	1.41	1.41	1.41	1.41
Salt	0.36	0.36	0.36	0.36
Methionine (99%)	0.10	0.10	0.10	0.10
Lysine	0.03	0.03	0.03	0.03
Mineral mix[a]	0.10	0.10	0.10	0.10
Vitamin mix[b]	0.10	0.10	0.10	0.10
Purified fish oil	-	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00
Calculated value				
Crude protein (%)	21.50	21.50	21.50	21.50
Ca (%)	0.95	0.95	0.95	0.95
P (%)	0.69	0.69	0.69	0.69
Available P (%)	0.41	0.40	0.40	0.40
Lys (%)	1.15	1.15	1.15	1.15
Met (%)	0.44	0.44	0.44	0.44
Metabolisable energy (kcal/kg)	3200	3200	3200	3200
FAT (%)	4.43	4.49	4.56	4.63
Fibre (%)	3.49	3.49	3.49	3.49
Ash (%)	6.40	6.40	6.40	6.40
Omega-3 (%)	0.04	0.05	0.05	0.05
Omega-6 (%)	1.08	1.08	1.08	1.08

[a]Provided per kg of complete diet: 37.5 mg Zn (as $ZnSO_4$); 37.5 mg Mn (as MnO_2); 37.5 mg Fe (as $FeSO_4{\cdot}7H_2O$); 3.75 mg Cu (as $CuSO_4{\cdot}5H_2O$); 0.83 mg I (as KI); and 0.23 mg Se (as $Na_2SeO_3{\cdot}5H_2O$).

[b]Provided per kg of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 IU of vitamin E, 2.55 mg of vitamin K_3, 3 mg of Thiamin, 7.5 mg of Rivoflavin, 4.5 mg of vitamin B_6, 24 ug of vitamin B_{12}, 51 mg of Niacin, 1.5 mg of Folic acid, 0.2 mg of Biotin and 13.5 mg of Ca-Pantothenate.

TABLE 4 Feed composition of broiler (as fed-basis)

Item	Finisher (22–35 days)			
	CON	TRT1	TRT2	TRT3
Ingredients (%)				
Corn	53.52	53.47	53.42	53.34
Soybean bran	28.15	28.18	28.20	28.25
Corn gluten meal	11.00	11.00	11.00	11.00
Wheat bran	3.00	3.00	3.00	3.00
Soyoil	1.26	1.24	1.21	1.19
Mono calcium phosphate	0.95	0.95	0.95	0.95
Limestone	1.47	1.47	1.47	1.47
Salt	0.36	0.36	0.36	0.36
Methionine (99%)	0.06	0.06	0.06	0.06
Lysine	0.03	0.03	0.03	0.03
Mineral mix[a]	0.10	0.10	0.10	0.10
Vitamin mix[b]	0.10	0.10	0.10	0.10
Purified fish oil	-	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00
Calculated value				
Crude protein (%)	20.00	20.00	20.00	20.00
Ca (%)	0.90	0.90	0.90	0.90
P (%)	0.62	0.62	0.62	0.62
Available P (%)	0.35	0.35	0.35	0.35
Lys (%)	1.06	1.06	1.06	1.06
Met (%)	0.38	0.38	0.38	0.38
Metabolisable energy (kcal/kg)	3200	3200	3200	3200
FAT (%)	4.12	4.19	4.25	4.32
Fibre (%)	3.31	3.31	3.31	3.31
Ash (%)	5.93	5.93	5.93	5.93
Omega-3 (%)	0.04	0.05	0.05	0.05
Omega-6 (%)	1.19	1.19	1.18	1.18

[a]Provided per kg of complete diet: 37.5 mg Zn (as $ZnSO_4$); 37.5 mg Mn (as MnO_2); 37.5 mg Fe (as $FeSO_4{\cdot}7H_2O$); 3.75 mg Cu (as $CuSO_4{\cdot}5H_2O$); 0.83 mg I (as KI); and 0.23 mg Se (as $Na_2SeO_3{\cdot}5H_2O$).

[b]Provided per kg of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 IU of vitamin E, 2.55 mg of vitamin K_3, 3 mg of Thiamin, 7.5 mg of Rivoflavin, 4.5 mg of vitamin B_6, 24 ug of vitamin B_{12}, 51 mg of Niacin, 1.5 mg of Folic acid, 0.2 mg of Biotin and 13.5 mg of Ca-Pantothenate.

2.5 | Analysing meat quality and organ weight

No sooner had the blood samples (1 bird/pen, 20 samples/treatment) collected than the birds were sent to the slaughterhouse to be killed. Immediately after the slaughter, breast muscle, liver, spleen, abdominal fat, gizzard and bursa of fabricius had been collected and weighed with care. Breast muscle samples were delivered to the laboratory within an hour for weight measuring purposes. One of the meat sensory properties, the meat colour was measured at three locations using a portable Konica Minolta CR-400 chroma meter (Konica Minolta) to determine lightness (L*), redness (a*) and yellowness (b*). The measurement of pH was done twice with an inserting glass electrode pH-meter (Testo 205; Testo). For

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TABLE 5 Fatty acid composition of experimental diets

Items	Grower				Finisher			
	CON	TRT1	TRT2	TRT3	CON	TRT1	TRT2	TRT3
C16:0 (Palmitic acid)	11.69	11.55	11.45	11.55	11.79	11.66	11.45	11.33
C18:0 (Stearic acid)	3.21	3.16	3.12	3.14	2.99	2.94	2.86	2.84
C16:1n7 (Palmitoleic Acid)	0.14	0.14	0.14	0.14	0.14	0.15	0.14	0.14
C18:1n9 (Elaidic acid)	28.84	28.47	28.19	28.41	28.41	28.04	27.48	27.22
C18:2n6 (Linoleic acid)	50.84	49.68	48.29	49.75	51.26	50.68	49.92	49.39
C18:3n3 (α-Linolenic acid)	3.22	3.15	3.11	3.11	2.68	2.62	2.50	2.50
C22:6 (DHA)	0.02	0.03	0.05	0.06	0.02	0.04	0.04	0.07
Others	2.04	3.82	4.65	3.84	2.71	3.87	5.61	6.51

Abbreviation: DHA, docosahexaenoic acid.

measuring the water holding capacity (WHC), a sample of 0.3 g was placed on a 125-mm-diameter filter paper and was pressed at 3000 psi for 3 min. We calculated the diameter of the initial sample outline area and the diameter left after pressing the same sample using the digitizing area sensor (MT-10S; M.T. Precision). Samples were weighed before and after cooking at 80°C using a water bath that gradually lowered the core temperature of the fillet to 72°C to determine the cooking loss (Hazrati et al., 2020). The procedure for calculating the percentage of drip loss was done following the methods described by Honikel (1998). Breast muscles were sliced into cubes and submitted to a trained sensory panel of nine members (Department of Animal Resource and Science, Dankook University, South Korea) for organoleptic examination. In four panel sessions, the meat samples were evaluated for tenderness, flavour and off-flavour. It was a blind taste test for the organoleptic evaluation. The score runs from 1 to 8, with 1 being the lowest and 8 being the highest. 0 was used to represent the lack of off-flavour.

2.6 | Statistical analysis

Each pen was served as an experimental unit, and the data were analysed using GLM (SAS Institute, 2014). For, blood lipid parameters and meat quality one-way analysis of variance were performed. For the linear and quadratic effects of increasing the dosage of purified FO in the diet, orthogonal polynomial contrasts were used. To further compare control and treatment means, the Duncan multiple range tests were used. As standard error of means (SEM) is the measure of variability in the data, the statistical significance of the acquired data was denoted by $p < 0.05$, while $p < 0.10$ was considered a trend.

3 | RESULTS

3.1 | Growth performance

The growth performance of broilers is presented in Table 6. On Day 21, the BW of broilers in TRT2 and TRT3 was higher ($p < 0.05$) than in control. Increasing doses of purified FO supplementation showed a linear ($p < 0.05$) increase in body weight on Day 21. In Phase 1, supplementation with 0.10% and 0.15% of FO linearly improved BWG in TRT2 and TRT3 group birds compared to CON. Increasing the dosage of FO supplementation increased the BWG linearly ($p < 0.05$) over the entire trial. FI and FCR showed no change in any of the phases.

3.2 | Organ weight and meat quality

The organ weight in Table 7 and the meat quality of the broiler are presented in Table 8. Increasing doses of FO increased breast muscle weight linearly ($p < 0.05$) with the highest value in TRT3. FO supplementation linearly decreased the liver weight ($p < 0.05$) in FO supplemented groups, where TRT1, TRT2 and TRT3 were lower than CON. Abdominal fat was linearly decreased in FO supplemented groups ($p < 0.05$). The supplementation of FO did not show any improvement in the spleen, bursa of fabricius, gizzard, breast muscle colour, WHC, drip loss and cooking loss. There was no significant difference observed in tenderness, flavour and off-flavour of broiler meat by supplementing purified FO in the broiler.

3.3 | Blood profile

The results of purified FO on broiler blood parameters are shown in Table 9. FO supplementation caused the lowest cholesterol ($p < 0.05$), and triglyceride ($p < 0.05$) in TRT3 than the CON group with a linear reduction. Low density lipoprotein (LDL) cholesterol was reduced by FO supplementation in TRT1, TRT2 and TRT3 than the CON group with a linear reduction ($p < 0.05$). FO supplementation increased RBC in the blood in TRT3 compared to the CON group but did not change any count on WBC in the broilers' blood. Lymphocytes tended to increase linearly ($p < 0.05$) by increasing FO supplementation.

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TABLE 6 The effect of purified fish oil supplementation on growth performance in broilers

Items	CON	TRT1	TRT2	TRT3	SEM	*p* Value		
						Group difference	Linear	Quadratic
BW (g)								
Day 7	153	157	156	154	1.58	0.07	0.74	0.39
Day 21	777[b]	785[ab]	796[a]	794[a]	6.07	0.004	0.002	0.66
Day 35	1746	1789	1793	1772	13.39	0.09	0.06	0.63
d7–21								
BWG (g)	624[b]	628[ab]	640[a]	640[a]	6.07	0.006	0.04	0.81
FI (g)	862	871	876	873	8.32	0.22	0.23	0.46
FCR	1.378	1.388	1.368	1.365	0.0161	0.16	0.40	0.70
D22 to 35								
BWG (g)	969	1004	997	1008	13.14	0.67	0.04	0.40
FI (g)	1812	1843	1838	1831	21.53	0.12	0.38	0.54
FCR	1.876	1.839	1.848	1.819	0.0278	0.25	0.25	0.75
D7 to 35								
BWG (g)	1593	1632	1637	1648	13.17	0.10	0.001	0.34
FI (g)	2674	2714	2714	2704	24.32	0.07	0.20	0.40
FCR	1.678	1.662	1.657	1.641	0.0165	0.40	0.12	0.92

Abbreviations: BW, body weight; BWG, body weight gain; CON, basal diet; FCR, feed conversion ratio; FI, feed intake; SEM, standard error of means; TRT2 basal diet + 0.05% FO; TRT3, basal diet + 0.1% FO; TRT4, basal diet + 0.15% FO.

[ab]Different superscripts within a row indicate a significant difference ($p < 0.05$) in response to CON vs. TRT1, TRT2 and TRT3 diets.

TABLE 7 The effect purified fish oil supplementation on organ weight in broilers

Items	CON	TRT1	TRT2	TRT3	SEM	*p* Value		
						ANOVA	Linear	Quadratic
Relative organ weight (%)								
Breast muscle	15.01[b]	16.2[ab]	16.52[ab]	17.02[a]	0.582	0.02	0.02	0.57
Liver	2.87[a]	2.53[b]	2.45[b]	2.4[b]	0.11	0.01	0.007	0.14
Spleen	0.17	0.14	0.14	0.14	0.012	0.17	0.25	0.39
Abdominal fat	1.26	1.28	1.22	1.12	0.094	0.003	0.01	0.02
Bursa of fabricius	0.18	0.16	0.16	0.17	0.017	0.25	0.44	0.28
Gizzard	2.03	1.99	1.93	1.96	0.059	0.08	0.11	0.34

Abbreviations: ANOVA, analysis of variance; CON, basal diet; SEM, standard error of means; TRT2 basal diet + 0.05% FO; TRT3, basal diet + 0.1% FO; TRT4, basal diet + 0.15% FO.

[ab]Different superscripts within a row indicate a significant difference ($p < 0.05$) in response to CON vs. TRT1, TRT2 and TRT3 diets.

4 | DISCUSSION

The current study demonstrates that the supplementation of purified FO contributed positively to broiler growth performance. Previous studies, Mansoub and Yadollah Bahrami (2011) with 2% FO, Yang et al. (2006) with 0.04% FO, Jiang et al. (2007) with 3% FO, Farhoomand and Chekani-Azar (2009) with 2% FO, and Atteh and Leeson (1984) with fats made of oleic and palmitic acid, found improvements in BWG which is similar to our experiment. It has been shown that long-chain n-3 fats (2% FO) can improve BWG (Mansoub & Yadollah Bahrami, 2011). Fats are digestible to a greater extent when they are unsaturated (Farhoomand & Chekani-Azar, 2009; Lopez-Ferrer et al., 1999; Newman et al., 1998). Also, Tancharoenrat et al. (2014) reported higher ileal digestibility of palmitic acid and stearic acid in broilers. As tested purified FO has a higher concentration of palmitic acid and stearic acid, the positive growth

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TABLE 8 The effect purified fish oil supplementation on meat quality in broilers

Items	CON	TRT1	TRT2	TRT3	SEM	*p* Value ANOVA	Linear	Quadratic
Breast muscle colour								
Lightness (L*)	53.32	52.49	52.13	51.53	0.821	0.12	0.12	0.85
Redness (a*)	32.61	32.74	32.36	32.27	0.415	0.47	0.31	0.86
Yellowness (b*)	11.98	12.44	12.02	12.38	0.468	0.49	0.38	0.86
pH value	7.76	7.77	7.84	7.79	0.017	0.01	0.18	0.37
Cooking loss (%)	25.95	23.84	25.28	25.42	0.843	0.08	0.63	0.86
WHC (%)	56.54	52.85	51.65	57.22	2.844	0.18	0.95	0.19
Drip loss (%)								
d 1	1.20	1.41	1.68	1.78	0.256	0.13	0.09	0.84
d 3	3.71	3.75	4.25	3.79	0.443	0.12	0.15	0.08
d 5	4.51	4.86	5.45	5.08	0.388	0.09	0.21	0.38
d 7	5.72	6.57	6.75	6.85	0.470	0.12	0.15	0.50
Organoleptic test								
Tenderness	6.5	6.6	6.8	6.8	0.24	0.59	0.20	0.23
Flavour	4.2	4.3	4.2	4.4	0.24	0.34	0.31	0.74
Off-flavour	0.6	0.7	0.6	0.8	0.02	0.64	0.45	0.53

Abbreviations: ANOVA, analysis of variance; CON, basal diet; SEM, standard error of means; TRT2 basal diet + 0.05% FO; TRT3, basal diet + 0.1% FO; TRT4, basal diet + 0.15% FO; WHC, water holding capacity.

[a,b]Different superscripts within a row indicate a significant difference ($p < 0.05$) in response to CON vs. TRT1, TRT2 and TRT3 diets.

TABLE 9 The effect of purified fish oil supplementation on blood profile in broilers

Items	CON	TRT1	TRT2	TRT3	SEM	*p* Value ANOVA	Linear	Quadratic
Day 35								
Cholesterol (mg/dl)	146[a]	131[ab]	130[ab]	118[b]	7.67	0.03	0.04	0.89
Triglyceride (mg/dl)	26.72[a]	27[ab]	26.50[ab]	22[b]	1.313	0.02	0.01	0.65
HDL cholesterol (mg/dl)	95	93	98	84	5.025	0.07	0.38	0.28
LDL cholesterol (mg/dl)	43[a]	32[b]	26[b]	30[b]	4.08	0.008	0.007	0.03
WBC (10^3/µl)	32.55	32.88	34.37	35.12	3.026	0.55	0.50	0.95
RBC (10^6/µl)	1.86[b]	2.08[ab]	2.32[ab]	2.79[a]	0.214	0.01	0.11	0.19
Lymphocyte (%)	79.68	92.28	95.55	96.53	6.052	0.08	0.07	0.36

Abbreviations: ANOVA, analysis of variance; CON, basal diet; HDL, high density lipoprotein; LDL, low density lipoprotein; RBC, red blood cell; SEM, standard error of means; TRT2 basal diet + 0.05% FO; TRT3, basal diet + 0.1% FO; TRT4, basal diet + 0.15% FO; WBC, white blood cell.

[ab]Different superscripts within a row indicate a significant difference ($p < 0.05$) in response to CON vs. TRT1, TRT2 and TRT3 diets.

response might be due to better fat digestion. The Pinchasov and Nir (1992) studies also showed that supplementing PUFA in the diet did not alter FI in broilers, which was constant with the current findings. So, the growth performance might be a result of fat conversion into the body muscle and adipose tissue of broilers. Conversely, Lopez-Ferrer et al. (Lopez-Ferrer, Baucells, Barroeta, Galobart, et al., 2001; Lopez-Ferrer, Baucells, Barroeta, & Grashorn, 2001), Mansoub and Yadollah Bahrami (2011) and Poorghasemi et al. (2013) reported that broiler performance was unaffected by 4% of FO supplementation. Also, 2.5% and 5.0% FO did not improve broiler performance as reported by Agboola et al. (2021). The reason for FO supplementation's being ineffective was the reduction of FI in those studies. A significant reduction in FI may be due to excessive supplementation of FO, which could also be the cause of the fishy smell (Chashnidel et al., 2010). Also, different doses of FO can be a reason for the difference in BWG. The FO composition of the FO plays a vital role in

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the improvement of BWG in broilers. Increasing growth could be achieved by reducing the catabolic response induced by immune stimulation (Rymer & Givens, 2005).

Bou et al. (2004) reported that 2.5% of FO showed improvement in the breast meat harvest, which is similar to our experiment. In contrast with our study, Alparslan and Ozdoghan (2006) found that the breast muscle of broilers was unaffected by supplementing with 3% of FO. As breast meat weight is a proportional measurement of the live weight of broilers, the increase in breast meat was due to a proportional reduction of abdominal fat weight. Purified FO reduced abdominal fat linearly in our experiment. Chekani-Azar et al. (2010), Navidshad et al. (2010) and Panda et al. (2016) found similar reductions in abdominal fat by supplementing FO in broilers and sheep. The reason for the fat reduction could be due to omega-3 fatty acids reducing the fat deposition of cholesterol, triglycerides and LDL, thereby reducing the circulation of VLDL (Chekani-Azar et al., 2010; Pinchasov & Nir, 1992). Abdulkarimi et al. (2011) reported that liver weight reduction is related to fat deposition in the liver. As lipid components like cholesterol, triglycerides and LDL in blood and abdominal fat formation were reduced by purified FO supplementation, the reduced fat deposition in the liver is predictable.

Taugbol (1993) found that feeding growing–finishing pigs 0.05% and 0.2% of FO per day had no impact on their sensory quality. Adding 0.95% of FO per kg of feed as a growing–finishing pig diet had no effect on flavour of the fresh meat, according to Morgan et al. (1992). In this present study, the reason for no effect of FO supplementation on fresh broilers meat might be the use of a low dose of FO. The fishy flavour from FO supplementation is related to 20:5n-3 and a higher percentage of 20:5n-3 produces less oxidative stable meat (Nute et al., 2007). As we have used a low dose of purified FO, the 20:5n-3 might not increase to a great extent in broiler meat. S. I. Lee and Kang (2019) discussed that FO supplementation can reduce stress and lipid peroxidation by increasing superoxide dismutase and decreasing malondialdehyde.

As we found in our study, Mansoub and Yadollah Bahrami (2011) also reported that supplementing with 1% and 2% of FO reduced triglyceride levels and cholesterol levels in the broiler. Similarly, Chashnidel et al. (2010) found that supplementing with 3% FO decreased triglycerides, cholesterol and LDL in the broiler. Also, Newman et al. (1998) described that reduced plasma cholesterol and triglycerides in broilers are associated with saturated fatty acid intake. On the other hand, Jeun-Horng et al. (2002) did not find any change in cholesterol by supplementing 2% of FO in the broiler. Lower levels of cholesterol and triglycerides may result from decreased hepatic synthesis, an increase in synthetic enzyme activity, proximal beta-oxidation, and a decrease in LDL receptors in the liver (Belzung et al., 1993). Also, a reduction in total cholesterol may result in lower cholesterol levels. In addition, modifications in the fluid and composition of the cell wall of plasma may cause a diminution in cholesterol and triglycerides. Raj et al. (2010) explained that blood lipid components are co-related with the EPA and DHA deposition in animal meat. So, the EPA and DHA deposition in meat increased blood triglyceride, and cholesterol reduction is possible. DHA supplementation stimulates ACOX1 gene expression in the liver that reduces triglycerides synthesis by fat catabolism in the liver Jon Meadus et al. (2011).

Jameel and Sahib (2014) reported that 0.5% of FO increased RBC levels in the blood, which is consistent with our results. Seidavi et al. (2014) found a positive impact on immune response using 1.5% and 2% FO in broilers. RBC may improve because of increasing Omega-3, and Omega-6 PUFA as T-cell responses may differ depending on how they are affected (Dewille et al., 1981; Stulnig, 2000), as well as having physiological changes in metabolism, which contribute to modulating the biosynthesis process (Boudreau et al., 1991). Korver and Klasing (1997) reported omega-3 fatty acids of having anti-inflammatory and immunosuppressive properties that might influence poultry immune response by moderating the phospholipid composition of immune cells. Therefore, supplementing purified FO to broilers can have a positive impact on meat consumers by decreasing their risk of heart disease.

5 | CONCLUSION

In short, purified FO supplementation proved to improve body weight, desirable meat characteristics and safe blood lipid index with a positive immune status in broilers. Out of three doses, 0.15% FO supplementation brought the best result.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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