Effects of dietary fish oil supplementation in the diet on performance of Broiler chicks

EI Hassanein[1], Abdallah E. Metwally[2] & Hossam Eldin M Abd Elbaky[3*]

Dept. of Nutrition & clinical Nutrition, Faculty of Vet. Med., Zagazig University, Egypt

[1] Professor, Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Zagazig University, 44519 Sharkia, Egypt, Email: gmailehassanein119@gmail.com

[2] Professor, Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Zagazig University, 44519 Sharkia, Egypt, Email: drabdalla75@yahoo.com

[3] Research scholar, Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Zagazig University, 44519 Sharkia, Egypt, Email: vet7ossam@gmail.com

* Correspondence to
Hossam Eldin M Abd Elbaky
E-mail: vet7ossam@gmail.com

Abstract: *This work was carried out to investigate the effect of dietary supplementation of fish oil on growth performance, carcass quality, immunological and serum biochemical parameters, total antioxidant capacity, intestinal morphology, mortality ratio and economic efficiency measures of broiler chicks. A total of 150 (ross 308) one day old chicks was distributed into 5 groups each contains 3 replicates (10 chicks/ replicate) fed on Five experimental diets contains different level of oils (0 oil, 1(SBO & FO), 2 FO and 3% FO respectively), during the experimental period (5 weeks). four chicks from each replicate were used for analysis of the experimental chicks. The results revealed that supplementation of fish oil in diets of broiler chicks 1,2 and 3 % improve (P< 0.05) the body weight (Bw) and body weight gain (BWG) than control groups but the best value were observed in 1&3% fish oil group beside improvement of feed conversion ratio (FCR) in all groups fed fish oil than control groups. However, numerically increased feed consumption in 1% SBO fed group than other groups. Significant (P< 0.05) improvement of carcass characteristics and dressing percentage gradually with fish oil supplementation. The values of USFA and n-3 PUFA were significantly (P< 0.05) higher in the breast muscle of broilers fed with fish oil compared to the control groups. But, SFA significantly (P< 0.05) decreased with fish oil than control groups. No significant difference in n-6 PUFA value between SBO and FO groups. So, N-6:N3 ratio in breast meat decreased gradually with FO addition. Also, triglycerides, cholesterol, LDL and VLDL concentrations were significantly (P< 0.05) reduced by fish oil treatments, but serum HDL-c, total protein (TP), and globulin (GL) concentrations were significantly (P< 0.05) increased by using diets containing fish oil. Also, provision of fish oil Significantly (P< 0.05) improves immune response in broilers chicken against Newcastle virus vaccine through increase antibody titer and TLC with decreased mortality rate. Significant (P< 0.05) improvement of intestinal morphology with fish oil supplement were detected. The highest economic efficiency was recorded in group fed 1% FO followed by 1% SBO, 2% FO, 3% FO,0% oil gradually. from the present study, it could be concluded that the supplementation of fish oil in chick's diets significantly improve the growth performance, body composition, immune response, serum biochemical parameters, intestinal morphology, decrease the mortality rate and economically efficient at rate 1% addition.*

Keywords: **broiler, fish oil, growth performance, immunity, gut morphology and economic efficiency**

1. INTRODUCTION

Production of meat from poultry has increased regularly over the years, and this approach is expected to continue. Alternatively, genetic advancement in poultry strains and superior understanding of the nutrition help chicken to reach the market weight 2 kg at 35 days of age and the efficiency of converting feed into poultry products moreover continues to improve **Ravindran, (2013)**. Addition of different types of oils to produce meat enriched with functional n-3 PUFA. By this way, chicken meat becomes recommended for consumption to anyone who takes care of diet and health (athletes, children, the elderly). Also, give additional advantage of increasing bird's health **Kralik, et al. (2018)**. Most vegetable oils have a high omega-6 to omega-3 fatty acid ratio. Soybean oil takes an intermediate position with an omega-6 to omega-3 ratio **Doppenberg and Van der Aar (2017)**.in addition, various sources of n-3 PUFA such as flaxseed, fish oil, fish meal, marine algae and canola oil are fed in the diets of broilers as a source of n-3 PUFA **Coorey, et al., (2015)**.

ALA has less expressed positive effect on human health than EPA and DHA, and its efficiency of conversion to EPA and DHA in the human body is only 2–10% or even less. LNA serves as a precursor for synthesis of EPA and DHA. N-3 PUFA and balance of n-3 to n-6 fatty acids (approximately 2:1) in human diet is very important in reducing the incidence of lifestyle diseases such as coronary artery diseases, hypertension, atherosclerosis, peripheral artery diseases, alzheimer's disease, arthritis, cancer and diabetes. As well as some auto immune and inflammatory diseases **Chiu, et al. (2008)**.

The very long chain polyunsaturated fatty acids (PUFAs) (C18–C22) and n-3 Omega PUFAs are apparently widely accepted as a part of modern nutrition because of their beneficial effects on metabolism **Gogus& Smith (2010)**. The performance parameters of broilers fed FO was improved relative to birds fed the poultry fat diets **Chekani-Azar et al., (2009)**. **Saleh et al. (2009)** Showed that the supplementation of feeds with fish oil at 1.5% improved performance in broiler chickens. Also, positive effects on blood parameters and economic analysis **Alparslan, and Özdogan. (2006)**.in addition to improved carcass weight, dressing percentage **Elzobier et al. (2016)**, improvements in broiler immunity and recovery from immunological challenges (i.e., vaccinations, bacteria, and viruses) **Kidd, (2004)**. Improvements the GI tract morphological effects are influenced directly by the type of fat or indirectly by the amount of feed consumed **Aziza et al.(2014)**. Therefore, the aim of the current study was to investigate the improved broiler performance and health condition of birds through consumption of specific fatty acids, particularly at the right n-6:n-3 PUFA ratio and this will produce meat that is beneficial to human consumers.

2. Materials and Methods

The study was carried out at Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

a) Experimental birds, accommodation and management

A total unsexed one-day old (ross 308) broiler chicks were obtained from a commercial hatchery. On arrival the chicks were individually weighed (the initial average body weight was similar) then randomly allocated into 5 equal groups each consisting of 3 replicates of 10 birds in each. The feeding trial lasted for 5 weeks. All chicks were fed crumble diet in starter and grower period then pellet diet till the end of the experimental period where the feeding is ad libitum. The chickens were vaccinated according to vaccination programs. five experimental (isocaloric and is nitrogenous) diets divided into 3 phases were formulated to contain 0 oil, 1(SBO & FO), 2 FO and 3% FO to meet the nutrient requirements for broiler chicks according to **Aviagen, (2019)** and to contain different ratio of n-3 to n-6 fatty acids as Shawn in Table 1. The representative sample of all the feed ingredients were analyzed

according to SupNIR-2700.Metabolic energy was calculated according to **Janssen (1989)**. The diets were kept at cold dry place to prevent oxidative rancidity.

The growth performance was determined through weighing the daily feed consumption and the body weight for each cage after each period until 5 weeks. At the end of the feeding trial, the total feed intake, weight gain and the feed to gain ratio were determined. Also, four birds from each replicate at 5-wk-old used for sampling were weighed, slaughtered and eviscerated without a feed withdrawal period according to **Brake et al. (1993)** were, eviscerated carcass, Liver, heart, gizzard, spleen, bursa, thymus gland, breast, thigh, abdominal fat yields and whole evacuated intestine are weighed for calculating dressing percent. Weight of lymphoid organ (bursa of Fabricius, spleen and thymus) were taken at the end of experiment from the sacrificed birds of each group to calculate the relative organ weight. Humoral immune response for Newcastle virus antibodies was assessed by hemagglutination Inhibition for newcastle vaccine antibody titter according to **Anon., (1971**). Also, total leukocyte count of non-coagulated blood sample was measured at the end of trial using an automatic blood analyzer. The serum were analyzed for Total cholesterol according to **Naito& Kaplan, (1984)**, Triglyceride according to **Wahlefeld & Bergmeyer, (1974)**, Serum high-density lipoprotein-cholesterol (HDL-C) according to **Burstein & Scholnick, (1973)**, Serum low-density lipoprotein-cholesterol (LDL-C) according to **Friedewald, et al., (1972)**, Serum very low-density lipoprotein (VLDL), total serum protein according to **Grant et al., (1987)**, albumin according to **Doumas & Biggs, (1971)** and globulin according to **Doumas & Biggs, (1972)**. The serum levels of Total Antioxidant Capacity (TAC) and glutathione peroxidase (GSH-Px) were assayed according to the methods adopted by **Koracevic *et al.* (2001)**. The homogenized freeze-dried breast meat was analyzed for fatty acid composition according to the method described by **Folch et al. (1957)** and the fatty acid methyl esters were prepared as described by **Ichihara and Fukubayashi (2010)** through gas chromatography (GC). Histopathological examination for intestine where the representative samples from jejunum according to **Drury and Wallington, (1967)** to study intestinal wall thickness (MT), villous height, crypts depth CD, villous height: crypt depth ratio, goblet cell proliferation, villus width (VW), villus perimeter calculated as (2π × (average villi width/2) × VH) and villus surface area (VA) calculated as villus perimeter× VH. During experiment in each treatment mortality ratio were calculated. An economic analysis was conducted to compare the live production costs for birds reared on the various experimental treatments according to **El-Kerdawy, (1997)**.

b) Statistical Analysis

Mean, standard error and coefficient of variation for the previous data will be calculated using the standard statistical formula given by **Snedecor and Cochran (1994)**. The data will be analyzed by ANOVA one-way classification using completely randomized design to test the significance of difference between different treatment groups.

Table 1. ingredient and nutrient composition of the experimental diets

Ingredients	Starter					grower					finisher				
Feeding period(days)	0-10 days					11-24 days					25-35 days				
Groups	G 1	G 2	G 3	G 4	G 5	G 1	G 2	G 3	G 4	G 5	G 1	G 2	G 3	G 4	G 5
Yellow corn	59.71	57.10	57.00	55.10	52.70	65.00	62.74	62.71	60.48	58.17	71.42	69.11	69.07	66.84	64.60
Corn gluten meal 60% cp	12.20	9.66	9.66	6.58	3.89	15.55	12.73	12.73	9.91	7.22	17.85	15.10	15.10	12.30	9.58
Soybeenmeal 48% cp	23.32	27.60	27.70	31.79	35.96	14.93	19.12	19.15	23.32	27.40	6.45	10.62	10.6	14.80	18.85
Fish oil	0.00	0.00	1.00	2.00	3.00	0.00	0.00	1.00	2.00	3.00	0.00	0.00	1.00	2.00	3.00
Soybean oil	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Grouned limestone	1.87	1.85	1.85	1.86	1.82	1.70	1.65	1.65	1.60	1.64	1.59	1.59	1.59	1.57	1.55
mono-calcium phosphate	1.29	1.25	1.25	1.25	1.21	1.25	1.25	1.25	1.10	1.19	1.10	1.05	1.05	1.03	1.02
L-lysine	0.41	0.33	0.33	0.25	0.17	0.46	0.38	0.38	0.30	0.22	0.53	0.44	0.44	0.36	0.28
Dl- methionine	0.19	0.20	0.20	0.23	0.24	0.11	0.13	0.13	0.16	0.18	0.07	0.09	0.09	0.11	0.14
L-threonine	0.07	0.06	0.06	0.05	0.05	0.04	0.03	0.03	0.01	0.02	0.04	0.03	0.03	0.02	0.02
Vitamin mineral premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Common salts	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.33	0.35	0.35	0.35	0.35	0.35
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Antimycotoxin	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Sum	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutritive value															
CP	23.00	23.16	23.20	23.00	23.05	21.52	21.50	21.52	21.50	21.51	19.52	19.53	19.54	19.52	19.50
EE	2.69	3.56	3.56	4.44	5.31	2.84	3.74	3.74	4.62	5.50	3.07	3.94	3.94	4.82	5.69
ME	3000.16	3000.38	3000.23	3000.33	3000.61	3100.91	3100.32	3100.74	3100.15	3100.41	3200.36	3200.30	3200.72	3200.08	3200.77
Calcium	0.96	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87	0.87	0.79	0.79	0.79	0.79	0.79
Available Phosphorous	0.45	0.45	0.45	0.45	0.45	0.44	0.44	0.44	0.44	0.44	0.40	0.40	0.40	0.40	0.40
N-3:N-6 ratio**	1:31.81	1:14.96	1:3.56	1:1.83	1:1.20	1:37.65	1:16.31	1:3.86	1:1.98	1:1.30	1:45.06	1:17.79	1:4.18	1:2.13	1:1.4

* Premixes containing vitamins and minerals according to requirement for broiler chicks as recommended in **Aviagen, (2019)** produced by Multivita company.

** different ratio of n-3 to n-6 fatty acids calculated according to **Gunstone, (1996)**

3. Results

Table 2. Effect of fish oil supplementation in chicks' diets on growth performance

growth performance	Dietary fish oil supplementation%				
	0% oil	1% SBO	1 % FO	2% FO	3% FO
BW, g/bird	2253.15 ± 88.10 ^{b}	2355.89 ± 63.32 ^{ab}	2475.21 ± 69.26 ^{a}	2436.25± 31.04 ^{ab}	2513.33 ± 25.59 ^{a}
BWG, g/bird	2209.68 ±88.02 ^{b}	2312.42 ±63.33 ^{ab}	2431.71±69.17 ^{a}	2392.58 ±31.17 ^{ab}	2469.97 ±25.68 ^{a}
FI, g/bird	3247.10 ± 143.89	3341.89 ± 88.50	3271.70 ± 47.15	3229.60 ± 16.87	3227.50 ± 82.52
FCR	1.47±0.03 ^{a}	1.44 ±0.00 ^{a}	1.35 ±0.04 ^{b}	1.35 ±0.02 ^{b}	1.31 ±0.02 ^{b}

a, b, means ± standard error in the same row with different superscripts are significantly different (P<0.05).
BW=body weight, BWG=body weight gain, FI=feed intake, FCR=feed conversion ratio

The main effect of dietary fish oil level on growth performance of the broiler chicks is that final BW and BEG (P<0.05) increased with dietary fish oil treated groups but the best BW&BWG was recorded in groups 1% and 3% FO then 2% FO. No significant difference observed in FI between control and FO treated groups and the highest value recorded in SBO group. all treatments containing fish oil significantly reduced FCR compared to control treatment which does not have and the best FCR was recorded in 3% FO group.

T**able 3.** Effect of fish oil supplementation in chicks' diets on carcass traits

carcass traits	Dietary fish oil supplementation%				
	0% oil	1% SBO	1 % FO	2% FO	3% FO
Body weight, g	2241.67 ± 7.26 ^{e}	2358.33 ± 4.41 ^{d}	2500.00 ± 7.64 ^{c}	2550.00 ± 5.77 ^{b}	2573.33 ± 9.28 ^{a}
Carcass weight, g	1520.00 ± 2.89 ^{e}	1614.33 ± 2.33 ^{d}	1804.33 ± 7.22 ^{c}	1840.00 ± 7.64 ^{b}	1875.00 ± 5.77 ^{a}
Carcass%	67.81 ± 0.18 ^{c}	68.45 ± 0.08 ^{c}	72.17 ± 0.10 ^{b}	72.16 ± 0.16 ^{b}	72.86 ± 0.37 ^{a}
Liver weight, g	46.23 ± 0.99 ^{c}	55.60 ± 1.07 ^{bc}	63.13 ± 3.96 ^{ab}	61.18 ± 1.37 ^{ab}	67.23 ± 5.02 ^{a}
Heart weight, g	9.85 ± 1.30 ^{b}	11.17 ± 0.73 ^{ab}	11.77 ± 1.06 ^{ab}	14.00 ± 0.58 ^{a}	10.49 ± 1.85 ^{ab}
Spleen	3.04 ± 0.04	4.09 ± 1.37	2.80 ± 0.02	3.03 ± 0.03	3.22 ± 0.11
Gizzard weight, g	32.68 ± 2.18 ^{ab}	27.17 ± 1.17 ^{b}	32.19 ± 1.30 ^{ab}	34.59 ± 1.82 ^{a}	33.78 ± 2.04 ^{a}
Total fat weight,	64.78 ± 3.01 ^{a}	66.00 ± 9.50 ^{a}	48.31 ± 3.68 ^{b}	55.00 ± 0.58 ^{ab}	59.67 ± 0.88 ^{ab}
Bursa weight, g	0.84 ± 0.04 ^{b}	0.86 ± 0.05 ^{b}	1.02 ± 0.03 ^{ab}	1.07 ± 0.07 ^{ab}	1.20 ± 0.19 ^{a}
Thymus weight, g	5.77 ± 0.79	6.50 ± 0.29	7.48 ± 1.09	7.40 ± 0.20	7.58 ± 1.53
Intestine weight, g	57.58 ± 4.33 ^{b}	59.00 ± 2.08 ^{b}	79.67 ± 1.20 ^{a}	79.74 ± 1.03 ^{a}	77.67 ± 1.45 ^{a}
Pectoral muscle weigh, g	651.67 ± 7.26 ^{c}	703.33 ± 16.41 ^{b}	781.67 ± 7.26 ^{a}	795.00 ± 30.41 ^{a}	798.33 ± 4.41 ^{a}
Thigh with drum stick weight, g	616.67 ± 1.67 ^{c}	658.33 ± 8.82 ^{b}	742.33 ± 9.33 ^{a}	751.67 ± 21.67 ^{a}	753.33 ± 7.26 ^{a}

a, b, c,d,e Means ± standard error in the same row with different superscripts are significantly different (P<0.05).

The result of carcass traits revealed that the dressing percentage, breast and thigh yield were (P<0.05) highest with significant (P<0.05) reduced abdominal fat percent in the fish oil than control groups. Also, improvement of thymus weight with fish oil groups than control groups and bursa improved with 3% fish oil supplement but spleen weight didn't reveal any improvement with fish oil addition.

Table 4. Effect of fish oil supplementation in chicks' diets on Serum biochemical parameters and total Antioxidant Capacity

Serum biochemical parameters	Dietary fish oil supplementation%				
	0% oil	1% SBO	1 % FO	2% FO	3% FO
Triglycerides (mg/dl)	57.16 ± 1.96 [a]	48.74 ± 1.40 [b]	40.11± 0.82 [c]	39.08 ± 0.18 [c]	37.82± 0.73 [c]
Total cholesterol (mg/dl)	141.71 ± 6.98 [a]	131.92 ± 7.93 [a]	108.29 ± 1.69 [b]	100.22 ± 3.05 [b]	93.13 ± 2.00 [b]
HDL (mg/dl)	56.26 ± 0.67 [b]	58.99 ± 0.75 [ab]	60.42 ± 2.30 [ab]	59.66± 1.09 [ab]	61.85 ± 1.77 [a]
LDL (mg/dl)	71.45 ± 7.60 [a]	60.93 ± 7.24 [a]	38.04 ± 2.54 [b]	31.06± 3.78 [b]	22.20 ± 3.50 [b]
VLDL* (mg/dl)	11.43 ± 0.39 [a]	9.75 ± 0.28 [b]	8.02 ± 0.16 [c]	7.82± 0.04 [c]	7.56 ± 0.15 [c]
Cholesterol ester (mg/dl)	2.57 ± 0.16 [a]	2.25 ± 0.12 [a]	1.81 ± 0.07 [b]	1.68 ± 0.07 [b]	1.52 ± 0.07 [b]
Total protein (mg/dl)	3.24 ± 0.10 [b]	3.28 ± 0.09 [b]	3.66 ± 0.07 [a]	3.51 ± 0.09 [ab]	3.65 ± 0.06 [a]
Albumin (g/dl)	1.89 ± 0.07 [ab]	1.93 ± 0.04 [a]	1.69b ± 0.08 [c]	1.76 ± 0.05 [abc]	1.67 ± 0.07 [c]
Globulin(g/dl)	1.35 ±0.03 [b]	1.35 ± 0.09 [b]	1.97 ± 0.05 [a]	1.74 ± 0.13 [a]	1.98 ± 0.12 [a]
TAC (mM/l)	0.33 ± 0.01 [a]	0.29 ± 0.04 [a]	0.19 ± 0.01 [b]	0.15 ± 0.01 [b]	0.14 ± 0.02 [b]
GSH-Px (IU/mg)	48.99 ± 1.89 [b]	50.98 ± 4.87 [b]	77.79 ± 6.61 [a]	81.43 ± 3.61 [a]	82.68 ± 2.39 [a]

a, b, c Means ± standard error in the same row with different superscripts are significantly different (P<0.05).
HDL=high density lipoprotein, LDL=low density lipoprotein, VLDL=very low-density lipoprotein, TAC=total antioxidant capacity, GSH-Px= Glutathione peroxidase
*Serum very low-density lipoprotein (VLDL) = Triglyceride/5

Serum triglycerides, cholesterol, LDL and VLDL concentrations were significantly(P<0.05) reduced with fish oil treatments, but serum HDL-c concentrations were significantly increased by using of diets containing fish oil. Also, significant (P<0.05) increased serum content of total protein (TP) and globulin (GL) concentrations but decreased albumin (A) concentration. The concentration of TAC was significantly decreased (P < 0.05) in broiler groups fed diet supplemented with fish oil when compared with control group. Also, a significant increase (P < 0.05) in GSH-Px values.

Table 5. Effect of fish oil supplementation in chicks' diets on Newcastle vaccine antibody titter, total leucocyte count and mortality rate

	Dietary fish oil supplementation%				
Exp. period (weeks)	0% oil	1% SBO	1 % FO	2% FO	3% FO
Antibody titer1st day	9.67 ± 0.33	9.67 ± 0.33	9.67 ± 0.33	9.67 ± 0.33	9.67 ± 0.33
Antibody titer at 14-day age	3.33 ± 0.33	3.67 ± 0.67	4.67 ± 0.67	5.00 ± 1.00	5.00 ± 0.58
Antibody titer at 26-day age	3.00 ± 0.00 [c]	3.33 ± 0.88 [bc]	4.33 ± 0.33 [abc]	5.00 ± 0.58 [ab]	5.33± 0.33 [a]
Antibody titer at 35-day age	2.33 ± 0.88	3.33 ± 0.33	4.00 ± 0.58	4.00 ± 1.00	4.00 ± 0.58
Total leucocyte count	166.07 ±2.46 [c]	170.87 ± 3.01 [bc]	179.20 ± 3.81 [ab]	182.53 ± 3.59 [a]	185.80 ± 3.50 [a]
Mortality rate	13.87 ± 2.79 [a]	8.32 ± 4.81 [ab]	0.00 ±0.00 [b]	0.00±0.00 [b]	0.00±0.00 [b]

a, b, c Means ± standard error in the same row with different superscripts are significantly different (P<0.05).

Numerically antibody titer against Newcastle disease vaccine increased with fish oil supplement groups than control groups. TLC significantly(P<0.05) give best ratio in 2% and 3% fish oil group and in fish oil groups than control groups. Also, significant(P<0.05) decreases mortality ratio with fish oil supplementation groups.

Table 6. Effect of fish oil supplementation in chicks' diets on intestinal morphology

Intestinal morphology	Dietary fish oil supplementation%				
	0% oil	1% SBO	1 % FO	2% FO	3% FO
VH (at 100X)	823.00 ± 7.51 ^{d}	896.00 ± 4.04 ^{c}	924.73 ± 17.41 ^{b}	1068.00 ± 4.62 ^{a}	1092.00 ± 2.89 ^{a}
CD (at 100X)	84.40 ± 2.83 ^{c}	93.60 ± 1.85 ^{b}	110.83 ± 2.88 ^{a}	115.37 ± 1.59 ^{a}	116.17 ± 1.79 ^{a}
VH/CD (at 100X)	9.78 ± 0.42 ^{a}	9.58 ± 0.15 ^{a}	8.36 ± 0.15 ^{b}	9.26 ± 0.17 ^{a}	9.41 ± 0.16 ^{a}
Mucosa thickness (at 40X)	418.50 ± 4.05 ^{d}	431.00 ± 2.16 ^{d}	485.63 ± 38.15 ^{c}	566.70 ± 1.26 ^{b}	1191.67 ± 2.85 ^{a}
wall thickness (at 40X)	492.67 ± 2.90 ^{d}	472.47 ± 1.55 ^{e}	556.27 ± 3.21 ^{c}	722.10 ± 2.33 ^{b}	1388.17 ± 4.21 ^{a}
VW (at 100X)	47.83 ± 1.17 ^{e}	73.28 ± 1.09 ^{d}	86.67 ± 2.87 ^{c}	101.27 ± 3.00 ^{b}	108.80 ± 2.64 ^{a}
villus perimeter	12.36 ± 0.19 ^{e}	20.63 ± 0.23 ^{d}	25.15 ± 0.35 ^{c}	33.99 ± 1.15 ^{b}	37.32 ± 0.81 ^{a}
villus surface area	10.17 ± 0.08 ^{e}	18.48 ± 0.15 ^{d}	23.24 ± 0.15 ^{c}	36.31 ± 1.39 ^{b}	40.75 ± 0.77 ^{a}

a, b, c, d,e Means ± standard error in the same row with different superscripts are significantly different (P<0.05).
VH= Villous height, CD =Crypt depth, VH/CD= Villous height: Crypt depth, VW= Villous width

significant improvement in intestinal morphology in fish oil based diets than control groups through significant improved villus height, crypt depth, mucosal thickness, wall thickness, villus width, villus perimeter and villus surface area.

Table7. Effect of fish oil supplementation in chicks' diets on breast muscle content of fatty acids (%)

fatty acids percent of breast muscle	Dietary fish oil supplementation%				
	0% oil	1% SBO	1 % FO	2% FO	3% FO
SFA	29.31 ± 0.14 ^{e}	54.63± 0.25 ^{a}	36.890 ± 0.17 ^{b}	33.87 ± 0.16 ^{c}	33.27 ± 0.16 ^{d}
USFA	65.71 ± 0.31 ^{a}	45.47 ± 0.22 ^{a}	62.20± 0.29 ^{b}	65.92 ± 0.31 ^{a}	66.23 ± 0.31 ^{a}
Total n-3 fatty acids	1.69 ± 0.14 ^{d}	2.19± 0.17 ^{d}	13.73± 1.14 ^{c}	20.03± 1.63 ^{b}	24.72 ± 1.99 ^{a}
Total n-6 fatty acids	36.55 ± 3.01 ^{a}	14.14 ± 1.09 ^{b}	15.49 ± 1.28^{b}	16.12 ± 1.31 ^{b}	14.06 ± 1.14 ^{b}
n-6:n-3 ratio	22.94 ± 1.89 ^{a}	6.87 ± 0.53 ^{b}	1.21 ± 0.10 ^{c}	0.86 ± 0.07 ^{c}	0.62 ± 0.05 ^{c}

a, b, c,d,e Means ± standard error in the same row with different superscripts are significantly different (P<0.05).
SFA =saturated fatty acid; UFA = unsaturated fatty acid, n-6=omega 6, n-3=omega 3

Significant increased levels of both USFA and N-3 in fish oil groups than control groups. So, significant decreased n-6:n-3 ratio gradually with increased fish oil in diet but the result of n-6 ratio that revealed no significance between SBO and FO groups but increased with 0% oil group. Also, decreased SFA in fish oil than control groups.

Table 8: Effect of fish oil supplementation in chicks' diets on economic efficiency of 35-day-old broiler chickens

	Dietary fish oil supplementation%				
	0% oil	1% SBO	1 % FO	2% FO	3% FO
Feeding cost of the obtained gain (LE)	18.52	18.93	19.33	19.71	20.40
Selling price of the obtained gain (LE/kg live weight)	23	23	23	23	23
Selling cost of obtained gain (LE)	50.82	53.19	55.93	55.03	56.81
Economic efficiency (EE) %	174.41	180.92	189.37	179.18	178.43

(LE)= Egyptian pound date of experiment= 1/3//2019

The result showed that feeding cost of the obtained gain (LE) had increased by increasing FO use but group without oil add have the lowest feeding cost. Also, selling cost of obtained gain (LE) had the same result. The highest economic efficiency was recorded in group fed 1% FO followed by 1% SBO, 2% FO, 3% FO,0% oil gradually.

Histopathological examination

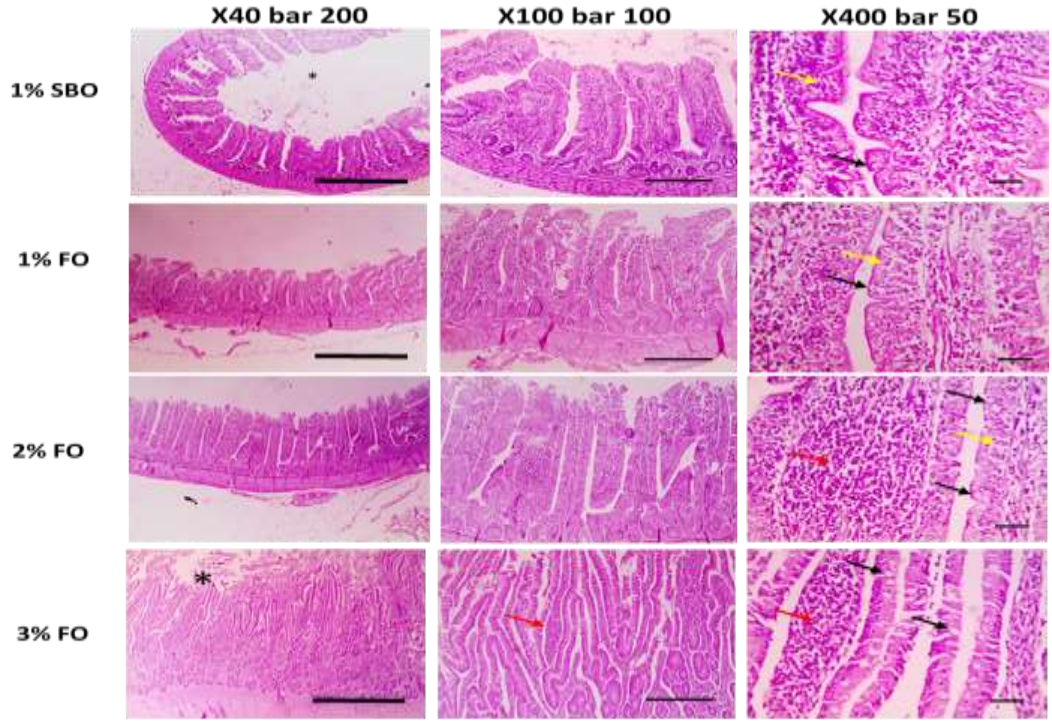

Fig.: Microscopic pictures of H&E stained jejunal sections showing normal villi, lamina propria and muscular coat in control group. Higher magnification X:400 show presence of few goblet cells (black arrow) along with epithelial vacuolization (yellow arrows). Meanwhile, duodenal sections from groups supplemented with ascending percentages of FO showing gradual increase in villous height, numbers and size of goblet cells (black arrow), lymphocytic cells population in lamina propria (red arrows) and decrease epithelial vacuolization (yellow arrows). (asterisks point to lumen of duodenum)

4. Discussion

a) Effect of fish oil dietary supplement on Growth performance

The main effect of dietary fish oil level on growth response of the broiler chicks is presented in Table 2. Final live weight ($P<0.05$) and weight gain ($P<0.05$) increased with dietary fish oil treated groups but the best BW&BWG was recorded in group 1% and 3% FO then 2% FO. This result agreed with previous findings who found that adding 3% fish oil improved broiler body weight, weight gain **(Elzobier et al. (2016) & EDIREES (2016))**. But this result disagreed with **Mirghelenj et al. (2009**) who observed that there was not any significant result of dietary treatments on Body Weight Gain (BWG) with diets contained 0-5% fish oil. No significant difference observed in feed consumptions between control and FO treated groups and this result agreed with **(Safamehr, et al (2008), Mirghelenj et al. (2009) & Chekani-Azar et al., (2009))**. But disagreed with **Saleh, et al. (2009)** who Showed that the supplementation of feeds with fish oil at 1.5% increased the FI where, a significant reduction in FI, were detected when the diet was supplemented with the maximum level of fish oil (6%). As presented by **Safamehr, et al (2008)** the birds fed 3% FO do the lowest FCR than other treatments. Also, all treatments containing fish oil significantly reduced FCR compared

to control treatment which does not have. Conversely **Hosseini-Mansoub and Bahrami (2011)** concluded that best FCR were recorded for the 1.5% FO dietary group, followed by 3% FO group. These results of growth performance might be due to the gorgeous content of omega-3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish oil. These fatty acids are well identified as essential nutrients for health and vital for numerous ordinary body functions and play a main role immune response, diet digestibility which stimulates growth **Saleh et al., (2009)**. Also, the role of n-3 PUFA in initiation of bile which improves fat digestion in the intestine, thus improve the efficiency of feed digestion and absorption **Jameel and Sahib, (2014)**. Moreover, omega-3 fatty acids of marine origin can influence skeletal muscle metabolism **Gingras et al. (2007).**

b) Effect of fish oil dietary supplement on carcass composition

Our result in table3 revealed that the dressing percentage, breast and thigh yield were ($P<0.05$) highest in the fish oil than control groups and this agreed with (**Ibrahim, et al. (2018), Elzobier et al. (2016) and Baiao and Lara, (2005**)) who presented that the use of oil or fat in diets for broilers may alter both the composition and the quality of the carcass. This may be due to the dietary fortification of omega-3 fatty acids that improves carcass by reducing the abdominal fat installation in broilers **Nafees & Pagthinathan, (2017)**. Also, ($P<0.05$) reduced abdominal fat percent as mentioned by **Chashnidel et al. (2010)** who indicated that the dietary omega-3 PUFA reduced the abdominal fat content of broiler chickens and this may be because marine source of omega-3 fatty acids has been found to be involved in the suppression of lipogenic genes in liver **Kaur and Sinclair, (2010)**. But **Safamehr, et al (2008**) investigated that significant differences were not found in weight of the carcass yield, abdominal fat, thighs, breast, liver, gizzard and heart among the treatments. The increased dressing and total edible parts of broilers groups that fed PUFA-enriched diet may be due to higher energy availability for muscle growth. also, the decrease in abdominal fat in these groups due to the shift in energy use for muscle growth rather than deposition in the abdominal cavity **Alagawany et al., (2019).**

c) Effect of fish oil dietary supplement on meat quality

Supplementation with fish oil (n-3 PUFA's) significantly increased levels of both USFA and N-3 matched to control diet as Shawn in table7. This result agreed with **(Newman, (2000) &Baiao and Lara, (2005))** who mentioned that in birds, body fat composition is related to the composition of the fat from the diet. Also, agreed with **Schreiner et al (2005)** who concluded that an optimized incorporation of LCω3PUFA into chicken meat can be gained by the use of marine oils of highest quality. Also, decrease SFA content of breast by increasing FO in the diet meat as concluded by **Chekani-Azar et al., (2009)**.

d) Effect of fish oil dietary supplement on serum biochemical parameters

Serum triglycerides, cholesterol, LDL and VLDL concentrations were significantly($P<0.05$) reduced with fish oil treatments, but serum HDL-c concentrations were significantly increased by using of diets containing fish oil. This result agreed with **(Chashnidel et al. (2010) & Hosseini-Mansoub and Bahrami (2011))**. This result may be related to the role of omega-3 fatty acid in reduction of triglycerides, high elimination of VLDL by liver and higher excretion of bile via feces which can also reduce the serum of cholesterol and triglycerides concentrations **Leaf and Weber, (1988)**.Also, significant ($P<0.05$) increased serum content of total protein (TP) and globulin (GL) concentrations but decreased albumin (A) concentration while **Hosseini-Mansoub and Bahrami (2011)** established that, with increasing levels of FO in the broiler diets, the total protein (TP), albumin (A) and globulin (GL) concentrations decreased. The improvement in protein with FO supplementation found here may be due to boosted immune response, and the antibodies

are proteinic in nature **Attia et al. (2020)**. The concentration of TAC was significantly decreased ($P < 0.05$) in broiler groups fed diet supplemented with fish oil when compared with control group. Also, a significant increase ($P < 0.05$) in GSH-Px values. These results agreed with **Qi, et al. (2010) &Ibrahim, et al. (2018)**) who concluded that enriching the diets with n-3 PUFA from FO clearly enhanced antioxidative status. Alike, **Bhattacharya et al. (2003)** who settled that n-3 PUFA scavenge H2O2 and lipid peroxides and thus can enhance the activities of the hepatic antioxidant enzymes.

d) The effect of fish oil dietary supplement on avian immune function

Our result revealed that weight of immune organ differs as a percentage of body weight with different fish oil supplement as Shawn in table 3 where improvement of thymus weight with fish oil groups than control groups and bursa improved with 3% fish oil supplement but spleen weight didn't reveal any improvement with fish oil addition. This result agreed with **Wang, et al. (2000**) who finished that at 4 wk, the chicks fed the three PUFA-rich diets (SO, LO, and FO) had significantly developed weights of thymus, bursa, and spleen as a percentage of body weight compared with the chicks fed the diet with a moderate level of PUFA (AO). TLC significantly($P<0.05$) give best ratio in 2% and 3% fish oil group and in fish oil groups than control groups as Shawn in table 5. This result agreed with **Al-Khalifa et al. (2017)** who revealed that the consumption of n-3 PUFAs particularly the long chain (>18 carbon atoms) fatty acids have been shown to have a pronounced effect on the health and immune status and rework the phenotypes of immune cells of different species including humans, rats and poultry. Numerically antibody titer against Newcastle disease vaccine increased with fish oil supplement groups than control groups (table 5). This is possibly because fish oil significantly increases the activation and number of T lymphocytes in the body and hence the ability of body to resist disease. Furthermore, the effect of fish oil on eicosanoid (leukotriene) and interleukin levels **Kidd, (2004)**. This result agreed with **Saleh, et al. (2009)** who showed that the antibody titer against SRBC was affected significantly by the dietary addition of fish oil where the fish oil groups had higher antibody titers compared to control group, while the maximum titer was in group supplemented with 3% fish oil. Also, the serum IgG concentration in the chicks fed FO was 73% greater than in the chicks fed SO, 37% higher than in the chicks fed AO, and 40% higher than in the chicks fed LO at 8 wk **Ramakrishnan, et al. (2010)**. Moreover, fish oil-treated birds had significantly extra serum antibody (predominantly immunoglobulin M, IgM) to SRBC than the control group and the highest response to primary and secondary injections of SRBC after 7 days, were noticed for group 4 (4% FO), followed by 2% FO group. So, the results indicate that the adding of 2 % FO to broiler chick's diet may stimulate the advance of the immune response, while 4% level was not recommended because of probable off-flavors in the product **Hosseini-Mansoub and Bahrami (2011)**.

e) Effects of fish oil dietary supplement on mortality ratio

Our result showed that fish oil supplementation significantly($P<0.05$) decreases mortality ratio (table 5). But **Carragher et al. (2015)** showed that there was no effect of dietary treatment on the mortality of the broilers during the trial. Overall mortalities were 6.0%in the Control diet fed broilers and6.7% in the high ALA fed broilers. This may be due to supplementation of PUFAs, to poultry diets, has been strictly connected with immune regulatory effects on both the innate and adaptive immunity through various mechanisms **de Pablo *et al.*, (2002)** and lower dietary levels of fish oil may be advantageous for improvements in broiler immunity and recovery from immunological tasks (i.e., vaccinations, bacteria, and viruses) **Kidd, (2004)**.

f) Effects of fish oil dietary supplement on intestinal morphology

Our results in table 6 showed that significant improvement in intestinal morphology in fish oil-based diets than control groups through significant improvement of villus height, crypt depth, mucosal thickness, wall thickness, villus width, villus perimeter and villus surface area. But Aziza **et al. (2014**) showed that the control and camelina meal diets as a source of n-3 PUFA increased villus height, VH:CD, villus perimeter at the jejunum when compared to fish oil and camelina meal containing diets and no significant difference in villus width, surface area, and muscularis thickness between different groups. On the other hand, **Nain et al. (2012)** found with total n-3 PUFA may not be an accurate picture of what may have happened in the bird over a longer time period. These results may be because fish oil increase the absorption capacity of the intestine. Therefore, improve the growth and immunity of broilers fed these diets **Attia et al. (2020)**.

g) The economic evaluation of fish oil dietary supplement

Our result showed that feeding cost of the obtained gain and selling cost of obtained gain (LE) had increased by increasing FO use but groups without oil addition have the lowest feeding cost. But the highest economic efficiency was recorded in group fed 1% FO followed by 1% SBO, 2% FO, 3% FO,0% oil gradually. However, **Kidd, (2004**) concluded that switching dietary poultry oil with fish oil is currently not economically acceptable. In conditions where fish oil is cost competitive, its inclusion level must be supervised because a 2 to 3% level in the diet can yield fish taste in broiler meat. The economic estimation of adding fish oil was made with a purpose of observing the cost using the methods of cost efficiency, and to advise the outcomes of the experiment for the use of producer conditions. The highest gross margin in the treatment groups was observed in the group 2% FO and then the group without fish oil supplementation fallowed this group, although, the lowest gross margin in the treatment groups was seen in group 4% FO **Alparslan, and Özdogan. (2006)**. Adding 3% fish oil enhanced production efficiency factor were significantly affected **Elzobier et al. (2016)**.

5. Conclusion

The supplementation of fish oil in chick's diets significantly improve the growth performance, body composition, meat quality, immune response, serum biochemical parameters, intestinal morphology, decrease the mortality rate and economically efficient at rate 1% addition.

6. Acknowledgments

The authors thank the *Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Zagazig University* for providing the facilities for carrying out this work.

7. REFERENCES

[1] *Alagawany, M., Elnesr, S. S., Farag, M. R., El-Hack, A., Mohamed, E., Khafaga, A. F., ... & Dhama, K. (2019). Omega-3 and omega-6 fatty acids in poultry nutrition: effect on production performance and health. Animals, 9(8), 573.*

[2] *Al-Khalifa, H. (2017). Enrichment of poultry diets with Polyunsaturated Fatty Acids (PUFA) for human consumption. Science, 80(6), 741-752.*

[3] *Alparslan, G., & Özdogan, M. (2006). The effects of diet containing fish oil on some blood parameters and the performance values of broilers and cost efficiency. Int. J. Poult. Sci, 5(5), 415-419.*

[4] *Anon, Y. (1971). Methods for Examining Poultry Biologics and for Identification and Quantifying Avian Pathogens. National Academy of Science, Washington, DC., USA, 66.*

[5] *Attia, Y. A., Al-Harthi, M. A., & Abo El-Maaty, H. M. (2020). The effects of different oil sources on performance, digestive enzymes, carcass traits, biochemical, immunological, antioxidant, and morphometric responses of broiler chicks. Frontiers in veterinary science, 7, 181.*

[6] *Aviagen. 2019. Ross 308 Broiler Nutrition Specifications. Aviagen Ltd., Newbridge, UK.*

[7] *Aziza, A. E., Awadin, W. F., Quezada, N., & Cherian, G. (2014). Gastrointestinal morphology, fatty acid profile, and production performance of broiler chickens fed camelina meal or fish oil. European Journal of Lipid Science and Technology, 116(12), 1727-1733.*

[8] *Baiao, N.C., and Lara, L.J. 2005. Oil and fat in broiler nutrition. Brazilian journal of Poul Sci. v.7/n.3/ 129-141.*

[9] *Bhattacharya, A., Lawrence, R. A., Krishnan, A., Zaman, K., Sun, D., & Fernandes, G. (2003). Effect of dietary n-3 and n-6 oils with and without food restriction on activity of antioxidant enzymes and lipid peroxidation in livers of cyclophosphamide treated autoimmune-prone NZB/W female mice. Journal of the American College of Nutrition, 22(5), 388-399.*

[10] *Brake, J., Havenstein, G. B., Scheideler, S. E., Ferket, P. R., & Rives, D. V. (1993). Relationship of sex, age, and body weight to broiler carcass yield and offal production. Poultry science, 72(6), 1137-1145.*

[11] *Burstein, M., & Scholnick, H. R. (1973). Lipoprotein-polyanion-metal interactions. In Advances in lipid research (Vol. 11, pp. 67-108). Elsevier.*

[12] *Carragher, J. F., Mühlhäusler, B. S., Geier, M. S., House, J. D., Hughes, R. J., & Gibson, R. A. (2015). Effect of dietary ALA on growth rate, feed conversion ratio, mortality rate and breast meat omega-3 LCPUFA content in broiler chickens. Animal production science, 56(5), 815-823.*

[13] *Chashnidel, Y., Moravej, H., Towhidi, A., Asadi, F., & Zeinodini, S. (2010). Influence of different levels of n-3 supplemented (fish oil) diet on performance, carcass quality and fat status in broilers. African Journal of Biotechnology, 9.(5).*

[14] *Chekani-Azar, S., Farhoomand, P., & Shahryar, H. A. (2009). Dietary fat type alters performance and quality of meat in male broiler. World Poultry Science Association (WPSA), 2nd Mediterranean Summit of WPSA, Antalya, Turkey, 4-7 October 2009, 539-543.*

[15] *Chiu, C. C., Su, K. P., Cheng, T. C., Liu, H. C., Chang, C. J., Dewey, M. E., ... & Huang, S. Y. (2008). The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: a preliminary randomized double-blind placebo-controlled study. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 32(6), 1538-1544.*

[16] *Coorey, R., A. Novinda, H. Williams and V. Jayasena. 2015. Omega-3 fatty acid profile of eggs from laying hens fed diets supplemented with chia, fish oil, and flaxseed. J. Food Sci. 80: S180–S187.*

[17] *de Pablo, M. A., Puertollano, M. A., & de Cienfuegos, G. A. (2002). Biological and clinical significance of lipids as modulators of immune system functions. Clin. Diagn. Lab. Immunol., 9(5), 945-950.*

[18] *Doppenberg, J., & Van Der Aar, P. J. (2017). Facts about fats: a review of the feeding value of fats and oils in feeds for swine and poultry. Wageningen Academic Publishers.*

[19] *Doumas, B. T., & Biggs, H. G. (1972). Standard methods of clinical chemistry. Academic Press, Chicago, 7, 175-189.*

[20] *Doumas, B. T., Watson, W. A., & Biggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. Clinica chimica acta, 31(1), 87-96.*

[21] *Drury, R. A. B., Wallington, E. A., & Cameron, S. R. (1967). Carleton's Histological technique. 4th ed. Oxford University Press, New York, pp 151, and pp 242–245*

[22] *EDIREES, M. A. M. (2016). Effect of Feeding Diet Contained Fish Oil on Broilers Performance and Blood Profile (Doctoral dissertation, Sudan University of Science and Technology).*

[23] *El-Kerdawy, D. M. A. (1997). Olive pulp as a new energy source for growing rabbits. Egyptian J. Rabbits Sci, 7, 1-12.*

[24] *Elzobier, M., Ibrahim, M. T. E., & Elbashier, O. M. (2016). Effects of dietary inclusion of fish oil on broiler performance and feed utilization. Int J Sci Technol Res, 5, 77-89.*

[25] *Folch, J., Lees, M., & Stanley, G. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. Journal of biological chemistry, 226(1), 497-509.*

[26] *Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry, 18(6), 499-502.*

[27] *Gingras, A. A., White, P. J., Chouinard, P. Y., Julien, P., Davis, T. A., Dombrowski, L., ... & Thivierge, M. C. (2007). Long-chain omega-3 fatty acids regulate bovine whole-body protein metabolism by promoting muscle insulin signalling to the Akt–mTOR–S6K1 pathway and insulin sensitivity. The Journal of physiology, 579(1), 269-284.*

[28] *Gogus, U., & Smith, C. (2010). n-3 Omega fatty acids: a review of current knowledge. International Journal of Food Science & Technology, 45(3), 417-436.*

[29] *Grant, G. H., Sliverman, L. M., & Christenson, R. H. (1987). Amino acids and proteins; In: Tietz NW, editor. Fundamentals of Clinical Chemistry. 3rd ed. . Philadephia: WB Saunders Company; 1987 . pp. 328–30.*

[30] *Gunstone, F. D. (1996). Fatty acid and lipid chemistry. https://doi.org/10.1007/978-1-4615-4131-8*

[31] *Hosseini-Mansoub, N., & Bahrami, Y. (2011). Influence of dietary fish oil supplementation on humoral immune response and some selected biochemical parameters of broiler chickens. Journal of Agrobiology, 28(1), 67-77.*

[32] *Ibrahim, D., El-Sayed, R., Khater, S. I., Said, E. N., & El-Mandrawy, S. A. (2018). Changing dietary n-6: n-3 ratio using different oil sources affects performance, behavior, cytokines mRNA expression and meat fatty acid profile of broiler chickens. Animal Nutrition, 4(1), 44-51.*

[33] *Ichihara, K. I., & Fukubayashi, Y. (2010). Preparation of fatty acid methyl esters for gas-liquid chromatography [S]. Journal of lipid research, 51(3), 635-640.*

[34] *JAMEEL, J. Y. & SAHIB, A. M. 2014. Study of some blood parameters of broilers fed on ration containing fish oil. Journal of Biology, Agriculture and Healthcare, 4, 67-71.*

[35] *Janssen, W. M. M. A. 1989. European Table of Energy Values for Poultry Feedstuffs. Subcommittee Energy of the Working Group nr. 2. Beekbergen, The Netherlands: Nutrition of the European Federation of Branches of the World's Poultry Science; ISBN 90-71463-00-0.*

[36] *Kaur, G., & Sinclair, A. J. (2010). Regulation of gene expression in brain and liver by marine n-3 polyunsaturated fatty acids. Progress in Nutrition, 12(1), 24-28.*

[37] *Kidd, M. T. (2004). Nutritional modulation of immune function in broilers. Poultry science, 83(4), 650-657.*

[38] *Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., & Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. Journal of clinical pathology, 54(5), 356-361.*

[39] *Kralik, G., Kralik, Z., Grčević, M., & Hanžek, D. (2018). Quality of Chicken Meat. Animal Husbandry and Nutrition, 63.*

[40] *Leaf, A., & Weber, P. C. (1988). Cardiovascular effects of n-3 fatty acids. New England Journal of Medicine, 318(9), 549-557.*

[41] *Mirghelenj, S. A., Golian, A., & Taghizadeh, V. (2009). Enrichment of chicken meat with long chain omega-3 fatty acids through dietary fish oil. Research Journal of Biological Sciences, 4(5), 604-608.*

[42] *Nafees, M., & Pagthinathan, M. (2017). Dietary enrichment of broiler chicken with omega-3 fatty acids and beneficial role in human cardiovascular health: A Review. AGRIEAST: Journal Of Agricultural Sciences, 10(0), 27. doi: 10.4038/agrieast.v10i0.26*

[43] *Nain, S., R. A. Renema, D. R. Korver, and M. J. Zuidhof. 2012. Characterization of the n-3 polyunsaturated fatty acid enrichment in laying hens fed an extruded flax enrichment source. Poult. Sci. 91:1720-1732.*

[44] *Naito, H. K. (1984): Cholesterol. Kaplan A. et al., Clin.Chem.The C.V. Mosby Co. St Louis. Toronto. Princeton. 1194-11206 and 437.*

[45] *Newman, R. E. 2000. Modulation of avian metabolism by dietary fatty acids. PhD. Thesis, University of Sydney, Australia.*

[46] *Qi, K. K., Chen, J. L., Zhao, G. P., Zheng, M. Q., & Wen, J. (2010). Effect of dietary ω6/ω3 on growth performance, carcass traits, meat quality and fatty acid profiles of Beijing-you chicken. Journal of animal physiology and animal nutrition, 94(4), 474-485.*

[47] *Ramakrishnan, U., Stein, A. D., Parra-Cabrera, S., Wang, M., Imhoff-Kunsch, B., Juárez-Márquez, S., ... & Martorell, R. (2010). Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico. Food and nutrition bulletin, 31(2_suppl2), S108-S116.*

[48] *Ravindran, V. (2013). Poultry feed availability and nutrition in developing countries. Poultry development review, 60-63.*

[49] *Safamehr, A., Aghaei, N., Mehmannavaz, Y., & Branch, M. (2008). The Influence of Different Levels of Dietary Fish Oil on the Performance. Research Journal of Biological Sciences, 3(10), 1202-1207.*

[50] *Saleh, H., Rahimi, S. H., & KARIMI, T. M. (2009). The effect of diet that contained fish oil on performance, serum parameters, the immune system and the fatty acid composition of meat in broilers. Iranian Journal of Veterinary Medicine (International Journal of Veterinary research), 3(2), 69-75.*

[51] *Schreiner, M., Hulan, H. W., Razzazi-Fazeli, E., Böhm, J., & Moreira, R. G. (2005). Effect of different sources of dietary omega-3 fatty acids on general performance and fatty acid profiles of thigh, breast, liver and portal blood of broilers. Journal of the Science of Food and Agriculture, 85(2), 219-226.*

[52] *Snedecor, G.W. and Cochran, W.G. (1994). Statistical Methods, 8th edition, IOWAState University Press, Amer, IOWA, USA, 217-268.*

[53] *Wahlefeld, A. W., & Bergmeyer, H. U. (1974). Methods of enzymatic analysis. Triglycerides determination after enzymatic hydrolysis. 2nd English ed., Academic Press, Inc, New York, 18-31.*

[54] *Wang, Y. W., Field, C. J., & Sim, J. S. (2000). Dietary polyunsaturated fatty acids alter lymphocyte subset proportion and proliferation, serum immunoglobulin G concentration, and immune tissue development in chicks. Poultry science, 79(12), 1741-1748.*