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Effect of Dietary Supplementation of Fish Oil as a Source of Omega-3 Fatty Acid on Growth Performance and Physiological Parameters of Growing Rabbits

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Authors' Contribution
YE designed the experiments, analyzed the data and revised the paper. SA revised the paper. AF performed the experiments and analyzed the data. WAM analyzed the data and wrote the paper. HM conducted the chemical analysis. All authors have read and agreed to the published version of the manuscript.

Key words
Blood, Fish oil, Growth, Malondialdehyde, Rabbit

ABSTRACT

This study aimed to assess the impact of fish oil levels in growing rabbit diets on growth performance and some blood parameters. One hundred and twenty APRI line rabbits, with an average live body weight of 914.4±12.35 g and an age of 6 weeks, were divided and randomly assigned into four experimental groups, each with 30 rabbits. Four experimental diets were formulated containing 0, 0.5, 1.0, and 1.5% fish oil, respectively. The results indicated that rabbits fed diets containing 1.0 and 1.5% fish oil had the highest final body weight, whereas those fed the control diet had the lowest final body weight. Also, the feed conversion ratio was the best in rabbits given fish oil diets containing 1.0 and 1.5% and the worst in those given the control diet. Serum total protein concentration was significantly increased (P<0.01) with increasing fish oil levels in diets. Malondialdehyde contents of serum, meat, and liver were significantly decreased with increasing fish oil levels in diets. It may be concluded that the inclusion of fish oil up to 1% of growing rabbits' diet enhanced the growth performance and improved their physiological status without any harmful effects on liver and kidney functions, under Egyptian environmental conditions.

INTRODUCTION

Rabbit production has many advantages such as high meat production, quick growth, small body size, and prolificacy. Comparable to pigs (16–18%) and beef (8–12%), rabbits can transform 20% of the protein they consume into palatable meat (Basavaraj *et al.*, 2011). It is generally recognized that rabbit performance can be enhanced by using feed additives in a safe manner (Ebeid *et al.*, 2013; Saleh *et al.*, 2013). Feed additives dietary supplemented in a little quantity led to some special effects. Fats and oils are one of the main sources of energy in diets (Leeson and Summers, 2005). Carbon, oxygen, and hydrogen are the basic component of dietary fatty acids, which are considered fatty acids that are saturated, monounsaturated, and polyunsaturated (Heird and Lapillonne, 2005). Important components of immune cell structure and eicosanoid production are polyunsaturated fatty acids (Ebeid *et al.*, 2008, 2011). The ratio and concentration of omega-6 and omega-3 fatty acids affect eicosanoid activity (Ebeid, 2011). Eicosanoids had an important role in changing the intensity and duration of the inflammatory response (Stulnig, 2004). They are resulted in enhancing vascular vasodilation and permeability, which improves inflammatory cytokines production. White blood cells release cytokines, which have a variety of impacts on lymphocytes and other immune cells in response to infection and damage. These actions act as regulators to the entire body. While omega-3 PUFAs have anti-inflammatory or less inflammatory qualities by reducing the secretion of pro-inflammatory eicosanoids and cytokines, omega-6 PUFAs have pro-inflammatory properties that enhance inflammatory eicosanoid, cytokine, and immunosuppression (Stulnig, 2004). Moreover, omega-3 supplementation can be targeted to specific pathways to prevent and alleviate intestinal diseases (Fu

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et al., 2021).

Fish oil contains a high concentration of linolenic acid (c18; 3omega-3), which is transformed by the body into additional n-3 fatty acids like docosahexaenoic acid (c22; 6omega-3) and eicosapentaenoic acid (c20; 5n-3), which could decrease cardiovascular diseases risk in humans (Gebauer *et al*., 2006). Fish oil is recommended for a healthy diet because it contains omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), precursors to eicosanoids that reduce inflammation throughout the body (Balwan and Saba, 2021). Many studies have concentrated on raising fatty acids levels in goods of animal origin (Parvarthy *et al*., 2016). Chekani-Azar *et al*. (2007) found that fish oil contains omega-3 fatty acids, which are an important factor in the diet for stimulating human and animal health. Omega-3 (PUFAs) is essential for supporting the prevention of cancer, inflammatory, autoimmune, and vascular diseases (El-Yamany *et al*., 2008). Ebeid *et al*. (2011) reported that a moderate amount (2% in diets) of n-3 PUFA improved the antioxidative state, decreased lipid peroxidation, promoted antibody response, and improved bone morphological characteristics while having no negative effects on the physical qualities of meat and growth performance in Japanese quail. Therefore, this experiment aimed to assess the impact of fish oil levels in growing rabbit diets on growth performance and some blood parameters.

MATERIALS AND METHODS

This study was carried out at the Rabbit Farm of Poultry Production Department, Faculty of Agriculture, Kafrelsheikh University during the period from December 2019 to February 2020. All experiment procedures concerning the use of animals were recognized by Kafrelsheikh University, Faculty of Agriculture's ethical committee following recommendations and rules for conducting rabbit nutrition studies (Fernández-Carmona *et al*., 2005).

One hundred and twenty APRI line rabbits, with an average live body weight of 914.4±12.35 g and an age of 6 weeks, were divided and randomly assigned into four experimental groups, each with 30 rabbits. To provide rabbits with the nutrients they need to grow, four experimental diets were created according to De Blas and Mateos (1998). The four diets were formulated to contain 0, 0.5, 1.0, and 1.5% fish oil, respectively. All diets had roughly the same quantities of microelements and were nearly iso-nitrogenous in energy. The components and chemical composition of these experimental diets are displayed in Table I. Fish oil chemical and fatty acid composition is 99.5% DM, 99% EE, 8000 kcal/ kg ME, 6.83% myristic (C14:0), 16.25% palmitic (C16:0), 6.57% palmitoleic (C16:1), 5.69% stearic (C18:1) 27.68% oleic (C18:1), 7.17% linoleic (C18:2), 2.95% linolenic (C18:3), 2.17% eicosenoic acid (C20:1), 3.99% arachidic acid (C20:0), 12.62% eicosapentaenoic acid (C20:5), 9.08% docosahexaenoic acid (C22:6), 31.76% total saturated fatty acids and 68.24% total unsaturated fatty acids, according to Hamed *et al*. (2020).

Table I. Composition and chemical analysis of experimental diets.

Ingredient	Control	Fish oil (%)		
		0.5	**1.0**	**1.5**
Berseem hay	30.5	29.8	28.5	26.8
Barley	30.0	25.6	19.5	13.4
Soybean meal (44%)	16.3	15.9	15.0	14.0
Wheat bran	19.4	24.4	32.3	40.7
Fish oil	0.00	0.50	1.00	1.50
Limestone	0.80	0.90	1.10	1.30
Di-Calcium phosphate	2.00	1.90	1.60	1.30
DL-Methionine	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30
Premix (1)	0.30	0.30	0.30	0.30
Anti-Fungi	0.10	0.10	0.10	0.10
Ati-Coccidia	0.10	0.10	0.10	0.10
Total	100	100	100	100
Chemical analysis (% as DM)				
Crude protein (CP)	17.00	17.04	17.04	17.03
Ether extract (EE)	1.62	2.19	2.82	3.45
Crude fiber (CF)	13.54	13.57	13.56	13.52
Lysine(2)	0.84	0.84	0.83	0.82
Methionine(2)	0.45	0.45	0.45	0.45
Calcium(2)	1.25	1.26	1.26	1.26
Phosphorus(2)	0.87	0.88	0.88	0.89
Metabolizable energy (kcal/kg)(2)	2272	2275	2273	2273

(1) Premix one kilogram contained: 150,000 UI Vit. A, 100 mg Vit. E, 21 mg Vit. K3, 10 mg Vit. B1, 40 mg Vit. B2, 15 mg Vit. B6, 100 mg Pantothenic acid, 0.1 mg Vit. B12, 200 mg Niacin, 10 mg Folic acid, 0.5 mg Biotin, 5000 mg Choline chloride, 0.3 mg Fe, 600 mg Mn, 50 mg Cu, 2 mg Co, 1 mg Se and 450 mg Zn. (2) Calculated.

Throughout the experiment, *ad libitum* of food and water were provided (6 to 14 weeks of age). The number of a dead rabbits, feed consumption, and live body weight were all recorded. Calculations were made for daily weight gain, feed conversion rate, and mortality rate. Also, calculations were made for the relative growth rate and performance index according to North (1984). Blood samples were taken from animals of treatment groups (6 rabbits each) to estimate some blood constituents. Blood serum total protein, glucose, triglycerides, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, and malondialdehyde (MDA) were calorimetrically determined by using commercial kits (Bio-Diagnosis Co., Cairo, Egypt), following the same procedures as explained by manufacturers.

The SAS (2000) General Linear Model Program was used to statistically evaluate the data. To find significant differences between the various quantities of fish oil, Duncan's multiple range tests were run (Duncan, 1955).

RESULTS

Growth performance

Table II represents growth performance as affected by dietary fish oil levels in growing rabbit diets. No significant differences could be detected in the initial body weight (6 weeks of age). At the end of the growing period, rabbits fed diets containing 1.0 and 1.5% fish oil had the highest final body weight, whereas those fed the control diet had the lowest final body weight (2527.2 and 2523.3 *vs.* 2253.3 g, P<0.001, respectively). As for daily weight gain, there was a significant increase in body weight with supplementing fish oil in diets. Rabbits fed fish oil diets significantly (P<0.001) increased daily weight gain by 19.5% (as average) in comparison to those fed a control diet. Generally, it could be observed that significantly higher relative growth rates were observed by supplementing fish oil in diets. Rabbits fed 1.5% fish oil diet had a significantly higher relative growth rate value, as compared to those fed a control diet (93.7 *vs.* 84.6%, P<0.001). Significant differences were found in feed intake. Rabbits fed a diet containing 0.5% fish oil recorded significantly the highest feed intake, while those fed a 1.5% fish oil diet had the lowest value (89.3 vs. 83.7 g, P<0.001). Also, the feed conversion ratio was the best in rabbits given fish oil diets containing 1.0 and 1.5% and the worst in those given the control diet (2.913 and 2.983 *vs.* 3.526 FI g / DWG g; P<0.001, respectively). Rabbits given 1.5% fish oil diet had the greatest performance index, while those given a control diet had the poorest value (87.1 *vs.* 64.1%; P<0.001, respectively). No mortality could be observed during the whole experimental period (6-14 weeks of age).

Blood serum parameters

Blood constituents of rabbits who were fed with different fish oil diets are presented in Table III. Serum total protein significantly increased (P<0.01) with increasing fish oil in diets. The incorporation of high doses of fish oil (1.0 and 1.5%) in diets increased blood glucose (P<0.05). Also, total antioxidant capacity (TAC) was significantly increased with increasing fish oil in diets, whereas rabbits fed a diet containing 1.5% fish oil were higher by 100% as compared with those fed a control diet. Liver function enzymes (serum AST and ALT) and kidney function markers (serum creatinine, and urea) did not significantly differ among treatments. The serum lipid profile of rabbits fed different fish oil diets is shown in Table III. Fish oil supplementation led to a significant (P 0.05 and P 0.01) reduction in serum triglycerides and total cholesterol. All tested levels of fish oil reduced significantly (P<0.05) the serum low-density lipoprotein (LDL) while increasing dietary fish oil levels had a significant positive impact on serum high-density lipoprotein (HDL).

Table II. Effect of fish oil level in growing rabbit diets on growth performance.

Parameters	Control	Fish oil level (%)			SEM	P value
		0.5	1.0	1.5		
Initial body weight (g)	913.9	914.4	915.0	914.4	12.35	0.9999
Final body weight (g)	2253.3[b]	2493.3[a]	2523.3[a]	2527.2[a]	21.78	0.0001
Daily weight gain (g)	23.9[b]	28.2[a]	28.7[a]	28.8[a]	0.374	0.0001
Feed intake (g/d)	84.2[b]	89.3[a]	85.5[b]	83.7[b]	0.878	0.0002
Feed conversion ratio	3.526[a]	3.171[b]	2.983[c]	2.913[c]	0.055	0.0001
Relative growth rate	84.6[b]	92.7[a]	93.5[a]	93.7[a]	0.874	0.0001
Performance index (%)[1]	64.1[c]	78.8[b]	84.9[a]	87.1[a]	1.722	0.0001
Mortality rate (%)	0	0	0	0	-	-

SEM, Standard error of means; [a, b, c] Means in the same row with different superscripts are significantly different (P<0.05). [1] Performance index = (Final live body weight (kg)/ Feed conversion ratio) x 100.

Table III. Effect of fish oil level in growing rabbit diets on some blood parameters.

Parameters	Control	Fish oil level (%)			SEM	P value
		0.5	1.0	1.5		
Total protein (g /dl)	5.41[b]	6.08[a]	6.29[a]	6.35[a]	0.137	0.0046
Glucose (mg/ dl)	49.5[b]	55.7[ab]	60.8[a]	61.3[a]	2.028	0.0246
TAC (U/mL)	0.04[b]	0.04[b]	0.07[ab]	0.08[a]	0.012	0.0862
Creatinine (mg/ dl)	1.28	1.21	1.28	1.20	0.064	0.8296
Urea (mg/ dl)	36.8	36.8	37.3	37.4	1.704	0.9930
AST (U/ml)	67.2	68.9	69.1	69.4	2.441	0.9392
ALT (U/L)	42.4	43.5	43.7	43.0	2.034	0.9834
Triglyserides (mg/ dl)	75.6[a]	69.7[ab]	61.7[bc]	53.3[c]	3.757	0.0138
Total cholesterol (mg/ dl)	40.4[a]	37.2[b]	36.1[b]	34.7[b]	0.833	0.0051
LDL-cholesterol (mg/ dl)	7.76[a]	7.18[ab]	6.86[b]	6.30[b]	0.232	0.0260
HDL-cholesterol (mg/ dl)	25.7[b]	27.1[ab]	28.4[a]	29.0[a]	0.666	0.0236

SEM, Standard error of means. [a, b, c] means in the same row with different superscripts are significantly different (P<0.05). TAC, total anti-oxidant capacity; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Malondialdehyde (MDA) values

The findings on the impact of dietary fish oil levels on the MDA content (an index of lipid peroxidation) in serum, meat, and liver are shown in Table IV. MDA contents of serum, meat, and liver were significantly decreased with raising dietary fish oil levels, whereas rabbits fed a diet containing 1.5% fish oil had significantly lower levels when compared to those fed the control diet.

Table IV. Effect of fish oil level in growing rabbit diets on MDA content.

Parameters	Con-trol	Fish oil level (%)			SEM	P value
		0.5	1.0	1.5		
Serum (mmol/mg)	25.15[a]	24.73[a]	22.98[a]	19.48[b]	0.720	0.0001
Meat (mmol/mg)	22.46[a]	21.15[ab]	19.62[b]	17.04[c]	0.711	0.0002
Liver (mmol/mg)	1.65[a]	1.43[a]	1.20[ab]	0.90[b]	0.138	0.0116

SEM, Standard error of means. [a, b, c] Means in the same row with different superscripts are significantly different (P<0.05).

DISCUSSION

The improvement in rabbits' productive performance fed fish oil could be due to enhanced diet digestibility, which encourages feed efficiency and growth (Saleh *et al*., 2009). The stimulation of bile, which improves fat digestion in the colon and increases the efficiency of feed digestion and absorption, may be responsible for this phenomenon (Jameel and Sahib, 2014). High levels of omega-3 PUFAs in fish oil cause significant changes in the gut microbiota, which might explain the health benefits of its use (Quin *et al*., 2020). In addition, fish oil exerts an inhibitory effect on a variety of bacteria. Omega-3 PUFAs could exert beneficial effects on the gut microbiota by decreasing the growth of *Enterobacteria*, increasing the growth of *Bifidobacteria*, and subsequently inhibiting the inflammatory response associated with metabolic endotoxemia (Cao *et al*., 2019). Also, an increase in n-3 PUFA in the diet can control metabolic function and pathophysiological processes, which can improve immunological and cardiovascular function as well as promote health (Stulnig, 2004; Ebeid, 2011). Similarly, Chekani-Azar *et al*. (2010) observed higher weight gain in broiler chicks who received dietary 1.5%, and 3% fish oil, as compared to those fed the control group. Ibrahim *et al*. (2018) reported that the administration of fish oil at 4% in diets of broiler chickens resulted in higher final body weight and protein efficiency and the best feed conversion ratios, as compared to the control group.

Serum total protein was significantly increased (P<0.01) by increasing fish oil concentration in the diets. This might be a result of these diets' increased CP digestibility (Amber, 2000). Liver function enzymes or kidney function did not show significant differences by dietary fish oil. This is in accordance with Alparslan and Ozdogan (2006), who found that dietary 2 or 4% fish oil did not significantly affect females' AST values. The decrease in lipid profile of rabbits fed fish oil could be attributed to the involvement of omega-3 fatty acids in the increased clearance of VLDL by liver peripheral tissues, decreased synthesis of triglycerides and apolipoproteins, and increased bile secretion in faeces (Jameel and Sahib, 2014), that could decrease cholesterol and triglycerides in serum. Similarly, Xiccato and Trocino (2003) found that the use of essential unsaturated fatty acids with suitable percentages between different acid types could decrease total cholesterol levels in depot fat and muscles. This effect results from energizing or inhibition of hepatic hydroxy-3-methylglutaryl-CoA reductase activity, an enzyme that manages the synthesis of cholesterol. In general, saturated fatty acids raise plasma LDL, which is very atherogenic through decreasing receptor-mediated cholesterol ingestion. While unsaturated fatty acids encourage plasma HDL production, which stimulates reverse cholesterol transport via an increase in scavenger receptor class B-1 expression and subsequent excretion of cholesterol by the liver (Nishimoto *et al*., 2009). Also, Saleh *et al*. (2009) found that plasma triglycerides and cholesterol decreased by raising the level of omega-3 fatty acids in the broiler diet, while dietary 4.5% fish oil decreased VLDL levels (Chashnidel *et al*., 2010). Moreover, Tag El-Din *et al*. (2017) observed that fish oíl supplementation (0.75 and 1.5%) in New Zealand White rabbit's diet significantly increased HDL and decreased LDL levels, as compared to the control diet.

Malondialdehyde contents of serum, meat, and liver were significantly reduced by raising fish oil levels in the diet. Omega-3 PUFA usage may have a calming impact in one of two ways: First, omega-3 PUFA may raise catalase levels in the cytoplasm and peroxisome, resulting in improved protection against free oxygen radicals (Ebeid, 2011). Second, the polyunsaturated fatty acid components of the membranes that had been harmed by oxygen free radicals like superoxide anions, hydrogen peroxide, and hydroxyl radicals may be substituted with the omega-3 PUFAs that have been supplemented (Ozgomen *et al*., 2000; Ebeid *et al*., 2008). Accordingly, Ibrahim *et al*. (2018) demonstrated that serum MDA concentration was reduced ($P<0.05$) in broilers when fed a fish oil and linseed oil diet, as compared to those fed a control diet. Similarly, in Japanese quail, Ebeid *et al*. (2011) observed that the inclusion of 2% fish oil reduced lipid peroxidation and improved the antioxidative status in meat and serum.

CONCLUSION

It may be concluded that the inclusion of fish oil up to 1% of growing rabbits diet enhanced the growth performance and improved their physiological status without any harmful effects on liver and kidney functions, under Egyptian environmental conditions.

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IBR approval

This study was approved by the Local Experimental Animals Care Committee's Ethics Committee and done according to the roles of Kafrelsheikh University, Egypt. (No. 4/2016EC).

Ethical statement

All experiment procedures concerning using birds were approved by the Kafrelsheikh University's Faculty of Agriculture's Ethics Committee.

Statement of conflict of interest

The authors have declared no conflict of interest.

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