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Effects of High Levels of Dietary Fish Oil on the Immune Response of European Sea Bass, *Dicentrarchus labrax*

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ABSTRACT

Lipids and their constituent fatty acids play essential and dynamic roles in the maintenance of optimum growth, feed efficiency and health of fish species. Lipids are primarily included in formulated diets to maximize their protein sparing effects. However, increasing the concentration of dietary fatty acids (EPA and DHA) in the fish diet can negatively affect fish immune response. In this study, some immune parameters were evaluated in European Sea Bass, *Dicentrarchus labrax* fed by a diet (50% crude protein) supplemented with fish oil at levels of 13-22%. In a 50-day feeding trial, 12 plastic tanks (80-L) were stocked with 18 fish per tank (~8g). The results showed that lysozyme and myeloperoxidase significantly decreased in fish fed diets with 16-22% fish oil inclusion. In conclusion, high-fat diets negatively affected some immune parameters of Sea Bass. Further studies are encouraged on the effect of dietary levels of fish oil on disease resistance.

Introduction

Dietary lipids and their constituent fatty acids play essential and dynamic roles in the maintenance of optimum growth, feed efficiency and health of fish species, especially for carnivorous fish as these species have limited ability to utilize carbohydrates as energy sources.

It is known that an increase in dietary lipid up to 18–19% improves protein utilization in Sea Bass (Kaushik 2002). Fish oil, the main source of oil in feed for Sea Bass, contains the two most important HUFA, eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). However, increasing the concentration of dietary EPA and/or DHA in the fish diet can negatively affect fish immune response and bacterial resistance (Fracalossi and Lovell 1994; Kiron et al. 1995; Yıldırım-Aksoy 2007). Fish immune response can also be modulated by a range of environmental factors such as temperature, oxygen, or chemicals.

European seabass (*Dicentrarchus labrax*) is one of most important commercial marine fish species widely cultured in Mediterranean. Fish in aquaculture conditions are often exposed to different stressors that might cause to significantly negative effects on fish welfare. Seasonal changes (Kavadias et al. 2004), culture conditions (Coz-Rakovac et al. 2005), stocking densities, and handling or harvesting (Vazzana et al. 2002; Roncarati et al. 2006; Marco et al. 2008) can cause stress that can increase risks of disease in Sea Bass. However, no study has been reported with respect to the dietary fish oil levels on the immune response of Sea Bass so far.

Therefore, the purpose of this study was to evaluate the effects of various dietary levels of anchovy fish oil on some immune parameters of European Sea Bass, *D. labrax*.

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Material and Methods

Diet formulations

Four practical diets were formulated to contain 49.9% crude protein with four lipid levels (13, 16, 19 and 22%) using fish meal and corn gluten as protein sources, anchovy oil as lipid source, wheat meal and starch as carbohydrate sources. The formulation and chemical composition of the experimental diets is displayed in Table 1.

Table 1. Feed ingredients, formulation and proximate composition of experimental diets (g/100g total diet).

	50/13	50/16	50/19	50/22
Fish meal[1]	62.0	62.0	62.0	62.0
Corn gluten[2]	10.0	10.0	10.0	10.0
Starch[3]	11.0	8.0	5.0	2.0
Wheat meal[4]	9.0	9.0	9.0	9.0
Fish oil[5]	6.0	9.0	12.0	15.0
Vitamin mix[6]	1.0	1.0	1.0	1.0
Mineral mix[7]	0.8	0.8	0.8	0.8
Cholin chloride[8]	0.2	0.2	0.2	0.2
Proximate Composition				
Crude Protein (%)	49.79	49.78	49.76	49.75
Crude Lipid (%)	12.97	15.95	18.93	21.92
Crude Ash (%)	9.10	9.08	9.06	9.04
Crude Fiber (%)	0.75	0.70	0.65	0.60
Nitrogen Free Extract (%)	25.38	22.48	19.59	16.69
Gross Energy (MJ/Kg)	21.29	21.97	22.64	23.32

All ingredients were thoroughly mixed in a food mixer (Dirmak Food Equipment, Turkey, model no: IBT-22) with warm water until a soft slightly moist consistency was achieved. The dough was then cold press extruded (PTM P6 extruder, Yalova, Turkey) to produce a 2 mm pellet. The moist pellets were broken up and sieved into proper pellet size and then fan-dried. All experimental diets were stored at -20°C until used.

Experimental facility and fish

The nutrition trial was carried out at the Beymelek Unit of Mediterranean Institute of Fisheries Research, Production and Training (AKSAM), Antalya, Turkey. Fish were obtained from a base population of juvenile grown at the hatchery of AKSAM. Before starting the experiment; all fish were acclimatized to the experimental conditions for a period of two weeks and fed twice a day with a sinking extruded commercial sea bass diet with 46% protein and 19% lipid. At the start of the trial, a total of 216 fish with initial mean weight of 8.50±0.01 g were randomly distributed into twelve 80-L tanks (18 fish per tank). Water parameters such as temperature, dissolved oxygen and pH were monitored daily by using OxyGuard Handy Gamma DO meter (DK-3460, Birkerod, Denmark; Deviation 9) and Testo pH meter (Testo, Vic., Australia), respectively. Photoperiod followed the natural course in the indoor system. During the course of the study, all experimental tanks were supplied with a sand filtered sea water flow of 7-l/min. Water parameters were measured as 7.90±0.02 pH, 35.0±0.10‰ salinity, 26.00±1.0°C temperature, and 5.95±0.60 mg/L dissolved oxygen. Fish were mass weighed in 2 weeks intervals and fish were deprived from feed one day prior to weighing. Experimental fish were hand fed ad libitum two times a day at 08:00 and 16:00 hours for 50 days.

Proximate composition

Standard AOAC (1998) procedures were followed for the analysis of crude protein, moisture, fiber and ash in fish whole body and the test diets. Dietary and whole body lipids were extracted according to the procedure of Soxhlet method. Nitrogen-free extract (NFE) was calculated by subtracting the sum values for crude protein, lipid, ash and crude fiber from 100. Gross energy content of the test diets and fish were calculated using the conversion factors of 23.7 kJ g^{-1} for protein, 39.5 kJg^{-1} for lipid and 17.2 kJ g^{-1} for carbohydrate (Brett and Groves 1979).

Blood Sampling

At the end of the feeding trial, two fish from each tank were sampled and anesthetized with 20 mg/l clove oil (Mylonas et al. 2005). Blood samples of six fish/groups were randomly collected from the caudal vein using a non-heparinized 2.5 ml syringe and put into a plastic biochemistry tubes. Then the blood serum was isolated by centrifugation at 2.142 × g for 10 min and used for lysozyme and myeloperoxidase activity.

Immunological Analysis

Lysozyme Activity

Serum lysozyme was assessed using the turbidometric assay (Ellis 1990). A suspension of 875 µl of *Micrococcus lysodeikticus* (Sigma, ATCC 4698) at a concentration of 0.2 mg/ml (in PBS) was added to 25 µl of serum samples, then measured spectrophotometrically at 530 nm after 0.5 and 4.5 min at 25°C, using a spectrophotometer. A unit of lysozyme activity was defined as the amount of serum causing a reduction in absorbance of 0.001 min^{-1}.

Myeloperoxidase Activity

Total myeloperoxidase (MPO) content was measured according to Quade and Roth (1997) and Sahoo et al. (2005) with slight modification. 30 µL serum was diluted with 370 mL of HBSS without Ca^{2+} or Mg^{2+} in Eppendorf tubes. 100 µL of 0.1 mg/mL (w/v) 3,3′,5,5′-tetramethylbenzidine dihydrochloride and 0.006% fresh hydrogen peroxide were added. The reaction was followed kinetically by measuring the increase of absorbance. Reaction velocities were determined as IU, defined as the amount of enzyme required to produce an 0.001 increase in absorbance per minute 0.5 mL of reaction mixture (ΔA 450/min/mL).

Table 2. Lysozyme and myeloperoxidase activity of sea bass fed different dietary lipid levels for 50 days.

		Average±SEM	95% Confidence Limit		Minimum	Maximum
			Lower	Upper		
Lysozyme (U/mL)	13%	441.67±22.97[a]	382.61	500.72	400.00	550.00
	16%	338.33±13.14[b]	304.54	372.12	300.00	375.00
	19%	326.67±12.36[b]	294.89	358.44	300.00	375.00
	22%	311.67±10.30[b]	285.19	338.15	275.00	350.00
Myeloperoxidase (U/L)	13%	96.61±5.75[a]	81.83	111.40	80.54	116.30
	16%	70.20±3.60[b]	60.94	79.46	61.01	80.00
	19%	67.00±2.37[b]	60.92	73.08	60.00	74.00
	22%	66.87±5.55[b]	52.61	81.12	41.94	78.00

Within a column, means with differing letters are significantly different ($P < 0.05$).

Statistical Analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA) and Tukey's multiple range tests to determine significant differences using $P < 0.05$.

Results

Immunological results are shown in Table 2. For the lysozyme activity (U/mL), the 95% confidence intervals have minimum/maximum values of 400.00/550.00, 300.00/375.00, 300.00/375.00 and 275.00/350.00 for dietary fish oil levels of 13%, 16%, 19% and 22%, respectively (Figure 1).

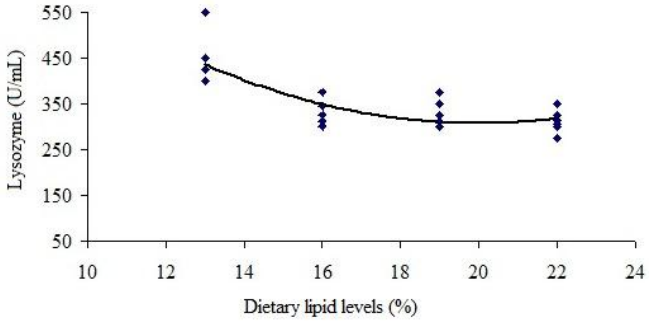


Figure 1. Lysozyme activity of sea bass fed different dietary lipid levels for 50 days.

For the myeloperoxidase activity (U/L), the 95% confidence intervals have minimum/maximum values of 80.54/116.30, 61.01/80.00, 60.00/74.00 and 41.94/78.00 for dietary fish oil levels of 13%, 16%, 19% and 22%, respectively (Figure 2).

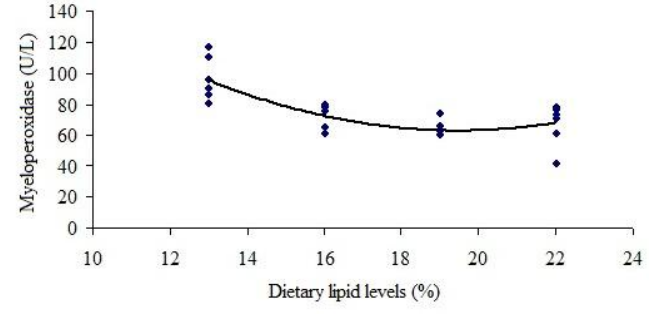


Figure 2. Myeloperoxidase activity of sea bass fed different dietary lipid levels for 50 days.

Lysozyme and myeloperoxidase activities were significantly highest in fish fed with the diet 13% added fish oil. Increasing dietary fish oil levels to 16% or higher (16 to 22%) significantly reduced lysozyme activity compared with those of fish fed with the control diet

Discussion

High levels of dietary fat have been reported to improve growth performance, feed utilization, and protein sparing with the reduction of nitrogenous losses and organic matter (Dias et al. 2001). However, the rate of mesenteric-perinephric and liver lipogenesis increases with an increase in the doses of this type of diet (Sargent et al. 2002). Additionally, lipid deposition plays an unfavorable role in terms of flavor, showing a negative effect on consumers' preference (Grigorakis 2007). It is also known that increasing the concentration of dietary lipid in the fish diet can increase lipid oxidation rates and consequently negative effect of health assessment index can be observed (Chaiyapechara et al. 2003).

In the present study, lysozyme and myeloperoxidase activities were determined in order to evaluate the effects of dietary fish oil levels on health and welfare conditions of Sea Bass. An exceptionally widespread defense molecule lysozyme is important for protection against fish pathogen because it directly activates polymorphonuclear leukocytes and macrophages or it promotes phagocytosis as an opsonin in freshwater and marine fish (Siwicki and Anderson 1993). Myeloperoxidase is contained in the polymorphonuclear neutrophils, monocytes, and macrophages (Klebanoff 1992). It participates in microbicidal activity and its activity informs fish neutrophil ability to kill the microorganisms (Siwicki et al. 1993).

The results of the present study showed that lysozyme and myeloperoxidase activities were significantly lowest in fish fed the diet with 16 to 22% dietary fish oil. Yıldırım-Aksoy et al. (2009) also reported that increasing dietary fish oil levels to 6 or 9% adversely affected some immune parameters of Channel Catfish, *Ictalurus punctatus*. Immunosuppressive effects of increasing dietary fish oil levels resulted with excessive levels of n-3 HUFAs. Suboptimal or excessive levels of n-3 and n-6 fatty acids, may adversely affect immune response and disease resistance of fish (Yıldırım-Aksoy et al. 2009). Marine oils

rich in n-3 PUFA are susceptible to oxidation (Kaushik 2002). Puangkaew et al. (2005) reported that the manipulated oxidative stress resulting from high dietary supplementation of n-3 HUFA could be linked to a general lowering of health indices of fish. Misra et al. (2006) also evaluated the use of different fatty acid levels (0.5, 1.0 and 2.0%) in a diet for *Labeo rohita* juveniles for 67 days and they stated that significantly lower lysozyme activity, serum total protein and globulin levels were recorded for the highest n-3 PUFA (2.0%) fed groups compared to lowest n-3 PUFA (0.5%) groups. Significantly lower survival was also recorded for the 2.0% n-3 PUFA fed groups.

The results in the present study showed that lysozyme and myeloperoxidase significantly decreased in fish fed with diets 16-22% fish oil inclusion levels. In conclusion, feeding European Sea Bass with high-fat diets resulted in negatively affected immune parameters. Further studies are encouraged to evaluate the effects of dietary levels of fish oil on disease resistance.

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