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High content of n-6 fatty acids in the flesh of farmed fish sea bass *Dicentrarchus labrax* L. and gilthead sea bream *Sparus aurata* L.: implications for the human health

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Abstract

The aim of this study was to present evidence for the health issues related to the fatty acid content in the edible flesh of farmed fish. The polyunsaturated fatty acids content of two widely farmed fish species in Europe, sea bass and gilthead sea bream, was 33.96% and 32.06% respectively. Linoleic acid (LA) was the dominant fatty acid and the n-3/n-6 ratio was 0.81 and 1.24, respectively. The dominance of LA and the increased n-6/n-3 ratio in the flesh in farmed fish of both species indicates the importance of fish feeds for the nutritional value of the final product. The fatty acids, especially n-3, are essential and important for human's health. Linoleic acid (LA) and Linolenic acid LNA in fish feeds cannot be transformed easily to n-6 and n-3 fatty acids in the flesh of marine fish. The fatty acid profile of fish is a determinant parameter for the health benefits of consuming fish. The data presented in this paper provide evidence of an urgent need to improve the fatty acid profile of these two widely consumed farmed fish in Europe.

Keywords: Fish quality, Farmed fish, Feeding, Sea bass, Sea bream

Introduction

Fish farming sector has annually developed at a rate of 8.8% during the last thirty years. Global consumption of fishery products reached 45 million tons in 2006, of which 45% originated from aquaculture (Wolmarans 2009). Accordingly, *per capita* consumption of fishery products has steadily been increasing in the last thirty years in all countries worldwide (Wolmarans 2009). This can be attributed to the improved economic level of the nations, to the reasonable prices of farmed fish, and to a positive consumers belief towards their nutritional value. Food and Agriculture Organization (FAO 2007) forecasted the global growth of fish farming sector in the very near future, particularly in Africa. This growth will be able to satisfy market demands for fishery products (two-folds increase of global population between 2006 and 2030), since production is estimated to reach 85 millions tons of fish for the year 2030.

Nutritional value of fishery products has been recognized by many studies (Keli et al. 1994; Sargent et al. 1999; Kris-Etherton et al. 2000; Kris-Etherton et al. 2003). Recent research findings regarding the quality and the nutritional value of farmed fish are worrying, due to the origin of the lipids and the qualitative composition of fish

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feeds in fatty acids, which are finally reflected in the flesh composition of fish (Phichova and Morkore 2007; Wassef et al. 2007; Maranghi et al. 2007; Dubois et al. 2007). Fish meal and fish oil substitution in fish feeds with alternative forms of nutrient sources [including more than 50% Linoleic acid (LA) and less than 7.8% 18:3 n-3 Linolenic acid (LNA) of total fatty acids] such as wheat flour, soybean meal, corn oil and sunflower oil, considerably alter the qualitative composition of lipids in fish flesh as well as the n-3/n-6 ratio (Phichova and Morkore 2007; Dubois et al. 2007; Martinez-Liorens et al. 2007).

The n-3/n-6 ratio is known to be important to physiology and health, not only to fish (Zhao and Etherton 2000; Cavallero et al. 2005), but also to humans (Kris-Etherton et al. 2003; Wolmaran 2009). Increased n-6/n-3 ratio (exceeding 5:1) leads to blood thrombosis and inflammatory reactions of various tissues (Sargent et al. 1999), whereas a low n-6/n-3 ratio has anti-thrombotic and anti-inflammatory properties, which are essential for the prevention of cardiovascular diseases (Kris-Etherton et al. 2003; Keli et al. 1994; Din et al. 2004). The balance between n-3 and n-6 fatty acids is also important for homeostasis, metabolism and normal development of the organism (Jumpsen and Clandinin 1995; Sargent 1997; Kris-Etherton et al. 2000; Pickova and Morkore 2007).

Contrary to wild marine fish species, farmed fish exhibit high ratio of n-6/n-3 fatty acids. This is usually attributed to a high dietary content of oleic acid (Torstensen et al. 2004; Lenas et al. 2010) or to other parameters such as genetic variability. Monoenoic fatty acids, mainly oleic acid, increase significantly in the muscle of farmed fish when fed with a diet rich in vegetable oil. The various sources of vegetable oil which can be found in aquaculture feed are frequently rich in oleic acid (Truswell and Choudhury 1998). In turn, the increased oleic acid in the fish feed can result in a higher accumulation of this fatty acid in the farmed gilthead sea bream. Fish fed with vegetable oil diets can show persistently high levels of linoleic and oleic acid in their flesh even for several months after switching to a fish oil diet (Fountoulaki et al. 2009).

The aim of this study was to assess the nutritional value by examining and comparing the fatty acid content in the edible flesh of farmed sea bass *Dicentrarchus labrax* L. and sea bream *Sparus aurata* L. of the same age (approximately 24 months), fed with the same type of fish feed and originating from the same aquatic environment.

Materials and methods

Fish

The study is based on recalculation of the fatty acid profile of farmed sea bass (Leans et al. 2011a) and gilthead sea bream (Leans et al. 2011b) of the same age (approximately 24 months), fed with the same type of fish feed and originating from the same aquatic environment. Twenty sea bass and twenty sea bream specimens with mean body weight of 466.87 g (± 32.19) and 419.36 g (± 30.20), respectively, were randomly selected in an intensive cage farm in Valtos Bay (located close to the mouth of River Kalamas, Ionian Sea, western Greece). Fish age was approximately 24 months. Additionally, samples of fish feeds were also collected. According to the label, fish feeds consisted of 45% proteins, 20% lipids, 14.5% carbohydrates, 9% ash, 1.5% fibres, 10% moisture, vitamins and trace elements. Raw materials were fish meal, fish oil, wheat flour, corn gluten, vitamins and minerals without any indication of inclusion percentage. The specimens were collected alive on May 2008. They were killed with thermal shock in 0 ˚C water and immediately transported in ice to the lab. Subsequently, fish were cleaned with tap water, scaled, de-headed, eviscerated and filleted after the skinning of the edible part of the flesh. All body parts of each fish were marked and stored in plastic bags in -30 ˚C before examination.

Determination of fatty acids

Flesh samples were de-frosted, chopped and subsequently the edible part of each fish was homogenized for one minute in a mechanical homogenizer with metallic blades under low temperature conditions (ice bath). On the very spot do at the feeds fish, after he addition 50% weight of water. Ten grams were finally used from the homogenate. Lipid extraction was performed using the Bligh and Dyer method (Bligh and Dyer 1959), as it was modified by Kinsella et al. (1977), using chloroform and methanol in a ratio of 2:1. The homogenate was centrifuged at 3000 rpm for lipids extraction; subsequently, the solution was removed, weighted and placed in a bottle for spin evaporation at 60 ˚C. The total weight of the lipids was calculated from using the formula: Total lipid= weight of lipid aliquot X volume of chloroform layer/volume of aliquot. Subsequently, fatty acids were methyl-esterified with a 12% boron trifluoride methanol solution (BF_3-MeOH) (Folch et al. 1957). Methyl-esters were obtained with normal hexane (Metchalfe et al. 1966). The analysis was performed using gas chromatography (GC-17A; Shimadzu, Kyoto, Japan) with capillary column and ionized flame detector (TRACE^{TM} TR-FAME GC Column, Thermo Fisher Scientific Inc.) and automatic sampler (HT 310A, HTA). Pure helium of 82 KPa flow, air (50 KPa) and hydrogen (60 KPa) were used for the analyses under the following conditions: initial temperature was 150 ˚C for 5 min, followed by a 5 ˚C/min pace until 170 ˚C for 10 min and then, 5 ˚C/min pace until 220 ˚C for 20 min. The identification of fatty acids (as methyl-esters) was performed by comparing the impressed peaks in special PC programme with Qalmix Fish (89-5550) and Methyl Dodecanoate (20-1200) fatty acid standards (Larodan Fine Chemicals AB).

Statistical analysis

Statistical analysis (mean values, standard deviation and proportions) was performed using Excel 2003 (Microsoft). T-test was applied after variability comparison by F-test.

Results

Total lipids (in 100 g of flesh) were 7.31 g (± 1.59) for the sea bass and 8.85 g (± 2.1) for the sea bream. Gas chromatography recognized forty to fifty fatty acids from which eighteen were identified, based on the standards (Table 1). Saturated fatty acids reached 20.60% of the total lipids in the sea bass and 20.14% in the sea bream (P=NS) and C16:0 was dominant in both species, followed by C18:0 and C14:0. Concerning mono-unsaturated fatty acids, they reached 33.79% in the sea bass and 39.45% in the sea bream *(P* < 0.001) and C18:1 was dominant in both species, followed by C22:1 and C16:1 in the sea bass and C16:1 and C22:1 in the sea bream.

PUFAs reached 34.14% in the sea bass and 32.02% in the sea bream (P=NS). In both cases, LA was dominant followed by C22:6n-3 (DHA) and C20:5n-3 (EPA). n-3 was increased in the sea bream compared to n-6, and the opposite trend was evident in the sea bass (Table 1). EPA/DHA ratio was 0.25 in the sea bass and 0.48 in the sea bream and n-3/n-6 ratio was 0.81 and 1.64, respectively. The results of the fatty acid analysis of the fish feeds are presented in Table 2. Total feed LA, n-6 and n-3/n-6 ratio were: LA=9.85%, n6=12.90% n3/n6=1.05 for sea bass, and LA=8.5%, n6=10.5%, n3/n6=1.45 for sea bream, respectively.

Table 1. Fatty acid profiles in the flesh lipids of farmed sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) (% of total fatty acids)[a]. Data calculated from Lenas et al. (2011 a, b).

Total fat and Fatty acid	Sea bass (n=20)	Sea bream (n=20)	P (*t*-test)
Total fat (g/100g flesh)	7.31 ± 1.59	8.85 ± 2.1	NS
C12:0	0.00 ± 0.00	0.00±0.00	NS
C14:0	2.75 ± 0.14	3.31±0.14	*
C15:0	0.33 ± 0.04	0.25±0.05	*
C16:0	13.81 ± 0.63	13.41±0.41	NS
C18:0	3.72 ± 0.15	3.18 ± 0.12	***
Total saturated	20.60 ± 0.94	20.14 ± 0.71	NS
C16:1 n-7 (9C)	3.97 ± 0.19	5.24 ± 0.16	***
C18:1 n-9 (9C)	19.56 ± 0.49	21.84 ± 0.55	***
C18:1 n-7 (11C)	3.15 ± 0.20	3.36 ± 0.15	NS
C20:1 n-9 (11C)	0.41 ± 0.06	4.02 ± 0.52	***
C22:1 n-9 (13C)	6.71 ± 0.14	5.00 ± 0.13	***
Total MUFAs	33.79 ± 1.03	39.45 ± 1.50	***
C18:2 n-6	18.02 ± o.12	11.13 ± 1.13	***
C18:3 n-3	2.08 ± 0.10	1.69 ± 0.10	***
C18:4 n-3	0.69 ± 0.06	0.74 ± 0.09	NS
C20:4 n-6	0.68 ± 0.04	0.66 ± 0.10	NS
C20:5 n-3	2.15 ± 0.08	4.47 ± 0.51	*
C22:4 n-6	0.12 ± 0.02	0.39 ± 0.08	**
C22:5 n-3	1.81 ± 0.06	3.76 ± 0.20	***
C22:6 n-3	8.60 ± 0.17	9.20 ± 0.22	***
Total PUFAs	34.14 ± 0.61	32.02 ± 2.38	NS
Total n-3 fatty acids	15.33 ± 0.44	19.85 ± 1.10	**
Total n-6 fatty acids	18.81 ± 0.18	12.17 ± 1.30	***
n-3/n-6 ratio	0.81 ± 0.02	1.64 ± 0.09	**
EPA/DHA ratio	0.25 ± 0.01	0.48 ± 0.04	***

[a]Mean ± standard deviation, NS=Non significant, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. Historical data, during the last decade, for Linoleic acid (LA) (%), total n-6 (%) and n-3/n-6 ratio in total lipids of wild and farmed sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*)

Parameter	Wild sea bass	Farmed sea bass	Wild sea bream	Farmed sea bream	Source
LA	3.2	5.7			
Total n-6	11.8	9.3			Alasalvar et al. (2002)
n-3/n-6 ratio	3.02	2.88			
LA			1.03	9.0	
Total n-6			9.3	11.86	Grigorakis et al. (2002)
n-3/n-6 ratio			3.09	1.92	
LA	2.26	7.21	1.57	5.56	
Total n-6	7.84	8.19	4.42	6.64	Orban et al. (2003)
n-3/n-6 ratio	3.02	2.77	2.73	3.63	
LA		7.0		7.43	
Total n-6		14.09		13.76	Ozden and Erkan (2007)
n-3/n-6 ratio		0.89		0.80	
LA		13.0		9.0	Average lipid composition in
Total n-6		13.80		9.90	sea bass and sea bream
n-3/n-6 ratio		0.80		1.55	aquaculture feeds

Table 3. Historical data on Linoleic acid (LA) (%), total n-6 and LA/total n-6 ratio of farmed gilthead sea bream at different feeding (Feed oil) regimes

Parameter	Group A	Group B	Group C	Reference
	Composition of feed			
	Fish oil and 60% SO[a]	Fish oil and 60% LO[b]	exclusively fish oil	
LA	23.68	10.36	5.53	
Total n-6	25.52	11.66	7.63	Izquierdo et al.
LA/total n-6 ratio	0.92	0.89	0.72	(2005)
LA	30.42	11.48	4.7	
Total n-6	31.27	12.87	7.72	Menoyo et al.
LA/total n-6 ratio	0.97	0.89	0.61	(2004)

[a] Soya Oil, [b] Lin Oil.

Discussion

The dominance of LA as well as the increased n-6/n-3 ratio in the flesh of both species of farmed fish indicated that fish feeds included increased amounts of plant oils as an alternative raw material for fish oil (Dubois et al. 2007). Results of the fatty acid analysis of wild fish from both species, collected from the same region (Lenas et al. 2011 a, b) demonstrated the superiority of wild fish in terms of the nutritional vaule of the fatty acid content. More specificly, the wild fish collected from the same region had lower levels of monounsaturates and a higher content of saturates and polyunsaturates, higher content of n-3, and higher ratios of n-3/n-6 and EPA/DHA.

A comparison between the fatty acid analysis of farmed and wild fish species, indicates that aquaculture feeds probably compromise the lipid composition of farmed fish, particularly the n-3 fatty acids, which are essential and important for human's health. Contrary to fresh water fish, LA and LNA in fish feeds cannot be transformed easily to n-6 and n-3 fatty acids in the flesh of marine fish. This is probably due to lack of necessary enzymes (D-5-6-Desaturase) which elongate the carbonic chain of fatty acids (Tocher 2003). This metabolic disability of marine fish leads to the inclusion of LA and LNA in flesh lipids, which are finally transferred to the human body through consumption. The main reason for the use of alternative sources of raw materials in fish feeds is the high cost of fish meal and fish oil due to increasing demand and limited production from wild fish stocks. This trend tends to increase as a convenient tool to reduce production cost in an intensely competitive market as emphasized by previous studies (Pichova et al. 2007; Dubois et al. 2007; Maranghi et al. 2007), and FAO estimates. During 2003, imports and consumption of fish meal and fish oil were decreased by 18% in the European Union, despite the increase of fish farming production (European Union 2005). This fact indicates that the alteration of fish feeds composition during the last decade has affected the taste of consumers.

A low content of fish oil and fish meal in aquaculture feeds may downgrade the nutritional in particular value and the beneficial content of n3 fatty acids of farmed fish and compromise the health benefits or even pose a health risk to consumers. Similar results have also been reported by other researchers (Alasalvar et al. 2002; Grigorakis et al. 2002; Orban et al. 2003; Martinez-Liorens et al. 2007; Ozden and Erkan 2007) (Table 2). The results from these studies show that the levels of LA have been increased up to 178-873% in farmed fish compared to wild fish. Experimental feeding trials in fish using plant oils (soybean oil and linseed oil) in fish feeds, showed that the addition of 60% soybean oil resulted in a 428-647% increased levels of LA in the flesh of fish compared to controls (exclusively fish oil). Similarly, the addition of 60% linseed oil resulted in a 187-244% increased levels of LA in the flesh of fish compared to controls (Robin, et al. 2003; Menoyo et al. 2004; Izquierdo et al. 2005; Martinez-Liorens et al. 2007). Additionally, the LA/total n-6 ratio ranged between 0.60 and 0.72 for the controls, instead of 0.92 to 0.97 (increased by 74.22%) in the soybean oil group. Similar results were also reported for the linseed group (Table 3). The study done by Hibbeln et al. (2004) on human populations in five western countries during a 39 years period of time (1961-2000) showed that increase in LA consumption might cause 100 times greater risk of mortality. Increased LA consumption, compared to the past centuries, may contribute to negative social phenomena such as aggressiveness, depression and increased cardiac diseases (Hibbeln et al. 2006). Accordingly, in order to prevent the health problems which arise from the consumption of fish with high n-6 fatty acids, the international community should enact quality assessment criteria and limits concerning both the qualitative composition of fish feeds and farmed fish, as well as effective control mechanisms.

In conclusion, the analyses of the flesh of farmed sea bass and sea bream showed that their LA contents were highly increased, which resulted in reduced n-3/n-6 ratio (below 2, compared to wild fish in which normally ranges between 2.5 and 6.8, Hibbeln et al. 2006). The results demonstrate that farmed fish are provided with plant nutrient sources rich in LA, in order to reduce production costs in a competitive market or due to temporary shortages in fish meal and fish oil. As a result, fish that are regarded as high n-3 food product lose their qualitative and functional properties, while are distributed in vast quantities to the market with altered and downgraded composition (increased n-6 levels).

In first years of global aquaculture development, fish meal and fish oil were two major components of aquaculture feeds. However, both fish meal and fish oil have recently become a costly ingredient. Plants can be an economic source of replacement for fish meal and fish oils in aquaculture diets, and the negative consequences of using vegetable oils on the fatty acid profile of farmed fish could at least partially be reduced by feeding fish with a fish oil based diet for a period prior to harvesting (Bell et al. 2003 a,b; Izquierdo et al. 2005). Nevertheless, there are some evidences to prove that the vegetable based aquaculture feeds can have long term effects on the fatty acid profile of farmed fish even for several months after switching from a vegetable to a fish oil based diet (Fountoulaki et al. 2009).

Compared to wild fish, farmed fish historically have always had higher contnent of n6 fatty acids. Both the total n6 content and the n6/n3 ratio of farmed fish increase with lowering the dietary fish oil and increasing the dietary vegetable oil (Table 3). In practise, fish farms may opt for the low cost option of using a vegetable oil based diet, in order to lower the cost, and this could explain the compromised fatty acid profile, in terms of the n3 content, of the farmed fish presented in the data we reviewed in the present paper. In turn, this can lead to the consumption of fish with compromised lipid quality which could potentially be dangerous for consumers who seek to consume fish on the basis of the health benefits of a low n3/n6 ratio diet (Asghari et al. 2011; Atkinson et al. 2011; Lucas et al. 2011; Sofi et al. 2011). Excessive consumption of n-6 fatty acids is regarded as a cause of social problems such as aggressiveness, depression and increased cardiovascular diseases (Hibbeln et al. 2004; Kris-Etherton et al. 2003). The data presented in this paper provide evidence of an urgent need to improve the fatty acid profile of these two widely consumed farmed fish in Europe.

References

Alasalvar C, Taylor KDA, Zumcov E, Shahidi F, Alexis M. 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. Food Chem 79: 145-150.

Asghari G, Mirmiran P, Eslamian G, Rashidkhani B, Asghari-Jafarabadi M, Azizi F. 2011. Inverse association of mediterranean diet with obesity and abdominal obesity: 6.7 years follow-up study. Iranian J Endocrinol Metabolism 13(1): 116.

Atkinson C, Whitley E, Ness A, Baker I. 2011. Associations between types of dietary fat and fish intake and risk of stroke in the Caerphilly Prospective Study (CaPS) Public Health, (in Press).

Bell JG, McGhee F, Campbell PJ, Sargent JR. 2003a. Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out". Aquaculture 218: 515-528.

Bell JG, Tocher DR, Henderson RJ, Dick JR, Crampton VO. 2003b. Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. J Nutr 133: 2793-2801.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917.

Cavallero MJ, Izquierdo MS, Kjorsvik E, Fernandez AJ, Rosenlund G. 2004. Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. J Fish Dis 27: 531-541.

Din JN, Newby DE, Flapan AD. 2004. Omega 3 fatty acids cardiovascular disease- fishing for a natural treatment. British Med J 328: 30-35.

Dubois V, Breton S, Linder M, Fanni J, Parmentier M. 2007. Fatty acid profiles vegetable oils with regard to their nutritional potential. Europ J Lipid Sci Technol 109: 710-732.

European Union 2005. Report 25 June 2005 for fish meal in the European Parlament.

FAO 2007. Conference Biennium 17-24 November 2007. Rome, Italy.

Folch J, Less M, Stanley GHS. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 457-509.

Fountoulaki E, Vasilaki A, Hurtado R, Grigorakis K, Karacostas I, Nengas I, Rigos G, Alexis MN. 2009. Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile. Recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. Aquaculture 289(3-4): 317-326.

Grigorakis K, Alexis MN, Taylor KDA, Hole M. 2002. Comparison of wild and cultured gilthead sea bream (*Sparus aurata*); composition, appearance and seasonal variations. Int J Food Sci Technol 37: 477-484.

Grigorakis K, Fountoulaki E, Vasilaki A, Mittakos I, Nathanailides C. 2011. Lipid quality and filleting yield of reared meagre (*Argyrosomus regius*). Int J Food Sc Technol 46: 711-716.

Hibbeln JR, Nieminen LR, Lands WE. 2004. Increasing homicide rates and linoleic acid consumption among five western countries, 1961-2000. Lipids 39: 1207-1213.

Hibbeln JR, Nieminen LRG, Blasbalg TL, Riggs JA, Lands WEM. 2006. Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. Am J Clin Nutr 83: 1483S-1493S.

Jumpsen J, Clandinin TM. 1995. Brain development: Relationship to dietary lipid and lipid metabolism. ADCS Press.

Izquierdo MS, Montero D, Robaina L, Caballero MJ, Rosenlund G, Gines R. 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead sea bream (*Sparus aurata*) feed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture 250: 431-444.

Keli SO, Feskens EJM, Kromhout D. 1994. Fish consumption and risk of stroke: The Zutphen study. Stroke 25: 328-332.

Kinsella JE, Shimp JL, Weihrauch J. 1977. Fatty acid content and composition of fresh water finfish. J Am Oil Chem Soc 54: 424-429.

Kris-Etherton PM, Taylor DS, Yo-Poth S, Huth P, Morlarty K, Fishell V. 2000. Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr 71(1): 179S-188S.

Kris-Etherton PM, Harris WS, Appel LJ. 2003. Fish consumption fish oil, ω-3 fatty acids, and cardiovascular disease. Arteriosclerosis Thrombosis Vascular Biol 23: 20-31.

Lenas D, Loghothetis P, Kanlis G, Nathanailides C. 2010. Comparison of fatty acids in the brains of wild and reared sea bass *Dicentrarchus labrax* L. and sea bream *Sparus aurata* L., and living in the same natural environment. Int Aquat Res 2: 135-141.

Lenas DS, Triantafillou DJ, Chatziantoniou S, Nathanailides C. 2011a. Fatty acid profile of wild and farmed gilthead sea bream (*Sparus aurata*). J Verbr Lebensm 6: 435-440.

Lenas DS, Chatziantoniou S, Nathanailides C, Triantafillou DJ. 2011b. Comparison of wild and farmed sea bass (*Dicentrarchus labrax* L) lipid quality. Procedia Food Sci (in press).

Lenas DS, Papadimitriou E, Bitchava C, Nathanailides C. 2011c. Fatty acid content and potential health benefits of consuming gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). Acta Alimentaria 40: 45-51.

Lucas M, Mirzaei F, O'Reilly EJ, Pan A, Willett WC, Kawachi I, Koenen K, Ascherio A. 2011. Dietary intake of n-3 and n-6 fatty acids and the risk of clinical depression in women: A 10-y prospective follow-up study. Am J Clin Nutr 93(6):1337-1343.

Maranghi F, Lorenzetti S, La Rocca, C, Tait S, Tassinari R, Frazzoli C. 2007. La valutazione del rischio - beneficio in sicurezza alimentare. Not Ist Super Sanita 20(11): 3-7.

Martinez-Liorens S, Tomas VA, Vicence MA, Pla TM, Jover CM. 2007. Effects of dietary soybean oil concentration on growth, nutrient utilization and muscle fatty acid composition of gilthead sea bream (*Sparus aurata* L.). Aqu Res 38: 76-81.

Menoyo D, Izquierdo M.S, Robaina L, Gines R, Lorez-Bote CJ, Bautista JM. 2004. Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soybean oils. British J Nutr 92: 41-52.

Metchalfe LD, Schmitz AA, Pelka JR. 1966. Rapid preparation of fatty acids esters from lipids for gas chromatography analysis. Annals Chem 38: 524-535.

Mourente G, Good JE, Bell JG. 2005. Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax* L.): Effects on flesh fatty acid composition, plasma prostaglandins E-2 and F-2 alpha, immune function and effectiveness of a fish oil finishing diet. Aqua Nutr 11: 25-40.

Orban E, Nevigato T, Di Lena G, Casini I, Marzetti A. 2003. Differentiation in the lipid quality of wild and farmed sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). J Food Sci 68(1): 128-132.

Ozden O, Erkan N. 2007. Comparison of biochemical composition of three aquacultured fishes (*Dicentrarchus labrax*, *Sparus aurata*, *Dentex dentex*). Int J Food Sci Nutr 59: 545-557.

Phichova J, Morkore T. 2007. Alternate oils in fish feeds. Europ J Lipid Sci Technol 109: 256-263.

Robin JH, Regost C, Arzel J, Kaushik SJ. 2003. Fatty acid profile of fish following a change in dietary fatty acid source: model of fatty acid composition with a dilution hypothesis. Aquaculture 225: 283-293.

Sargent JR. 1997. Fish oils and human diet. British J Nutri 78: 5-13.

Sargent J, Bell G, McEvoy L, Tocher D, Estevez A. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177: 191-199.

Sofi F, Abbate R, Gensini GF, Casini A, Trichopoulou A, Bamia C. 2011. Identification of change-points in the relationship between food groups in the mediterranean diet and overall mortality: an 'a posteriori' approach. Europ J Nutri (in Press).

Tocher DR. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci 11(2): 107-184.

Truswell AS, Choudhury N. 1998. Monounsaturated oils do not all have the same effect on plasma cholesterol. Europ J Clin Nutri 52: 312-315.

Wolmarans P. 2009. Background paper on global trends in food production, intake and composition. Annals Nutr Metabol 55: 244-272.

Wassef EA, Wahby OM, Sakr EM. 2007. Effect of dietary vegetable oils health and liver histology of gilthead sea bream (*Sparus aurata*). Aqua Res 38: 852-861.

Zhao G, Etherton TD. 2000. Polyunsaturated fatty acids in the food chain in the United States. Am J Clin Nutr 71(1): 179S-188S.