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Influence of partial substitution of dietary marine origin feedstuffs by a mixture of extruded pea seed meal and animal origin feedstuffs on fatty acid composition of fillet in sea bass (*Dicentrarchus labrax*)

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ABSTRACT

The aim of the study was to investigate how partial replacement of marine origin feedstuffs by a mixture of terrestrial origin (extruded pea meal, feather meal, spray-dried blood meal, soybean oil) feedstuffs affects the fatty acid composition of European sea bass fillet. Two experimental diets were formulated for the better growth of sea bass with an initial mean weight of 30 g in sea cages placed at 2500 m from the coast. The marine diet (MD) diet included 45% of marine origin feedstuffs, containing 30% of fish meal (herring), 10% of fish oil (anchovy) and 5% of krill meal, while terrestrial diet (TD) diet included 20% less of marine origin feedstuffs replaced by a mixture of terrestrial origin feedstuffs. The experimental diets did not affect growth performance and food conversion ratio in sea bass. The contents of EPA and DHA resulted lower in tissues of fish fed diet with the higher levels of terrestrial origin feedstuffs. Consequently, also the levels of total n-3 PUFA were lower in muscle of fish of this group; conversely total n-6 PUFA resulted in higher levels. According to the effect of treatments on both total n-6 and n-3 PUFA, the ratio of $\sum n-6/\sum n-3$ FA was higher in fillet of sea bass fed TD diet, maintaining anyway values sufficiently acceptable for a healthy diet according to Brenna et al.

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Introduction

One of the most important species for fish farming is sea bass (*Dicentrarchus labrax*). In the last years, particularly in the Mediterranean region, the production of this species is in rapid expansion. The global increase of aquaculture production has led to the partial replacement of fish meals by vegetable by-products in fish diet, considering that fish by-products can become an unpredictable and at high price feedstuffs and considering also that the catch for the production of meal, though regards species not of interest for human consumption, is anyway part of the marine 'food pyramid' (Turchini & Francis 2009). Furthermore, the European Union has re-introduced, by Regulation 56/2013 of February 2013, the use of animal meals (poultry and pork) in aquaculture after 12 years of suspension. Terrestrial animal by-products, such as meat and bone meal, spray-dried blood meal and hydrolysed feather meal, are considered to be potential alternative protein sources in aquatic

animal feed due to their high protein content and optimal amino acid profiles (Tacon 1993). These ingredients have been investigated in a wide range of fish carnivorous species (Wang et al. 2008; Xue et al. 2012).

In the last years, the consumers' demands in terms of quality are increased. Consumers require products which are safe, of consistent eating quality and healthy.

The health benefit of fish consumption, based mainly on the presence of high levels of n-3 highly unsaturated fatty acids (HUFA), especially eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), and on a low level of $\sum n-6/\sum n-3$ fatty acids (FA) ratio, makes seafood an important component of human nutrition (Lenas et al. 2011).

Considering that lipid composition of aquaculture feedstuffs can influence fatty acid composition of farmed fish, resulting often in lower level of n-3 polyunsaturated fatty acids (PUFA) and of the n-3/n-6 ratio (Lenas et al. 2011), fish nutrition plays an

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important role in Mediterranean aquaculture for a better product quality.

Some study demonstrated that the replacement of fish meal by a mixture of plant proteins did not have an effect on growth performance, but did affect the nutritional composition of the fillet in gilthead sea bream (Xue et al. 2006; Erolodogan et al. 2013). Similarly, Gouveia & Davies (2000) using a diet with a partial substitution of fish meal by pea seed meal found no difference in growth performance or diet utilisation in juvenile sea bass but observed differences in carcass composition.

A blend of terrestrial animal and vegetable protein sources could be a potential way to replace a higher level of dietary fish meal, providing a feed with good protein content at lower price.

The objective of the present study was to investigate the partial replacement of marine origin feedstuffs by a mixture of extruded pea seed meal and animal origin feedstuffs on fillet fatty acid composition in sea bass.

Materials and methods

Experimental design, animals and diets

A total number of 200 fish of the species *Dicentrarchus labrax* were used in the experiment obtained from a commercial fish farm in Southern Italy, Calabria. The initial mean weight of the fish was 30 ± 4.2 g and the mean length was 7.6 ± 0.8 cm. The fish were divided into groups of 50 and placed in experimental rearing sea cages with volume of 5 m^3 , placed at 2500 m from the coast. The net mesh size was 12 mm. The experiment was performed in duplicate. Successive weighing of the whole population of each cage was carried out every 30 days after anaesthetising the fish, following 24 h fasting. The experiment lasted for 12 months. The temperature during the experiment ranged from 18°C to 24°C (mean 21°C), the salinity was 38–39.2‰, the average dissolved oxygen was 6.5–8.24 mg/l and the pH was 8.2.

Two iso-energetic (about 15 MJ/Kg) and iso-proteic (40.12% and 39.92%, respectively for MD and TD diets) experimental diets were formulated with a constant lipid content of about 15%, 'marine' diet (MD) and 'terrestrial' diet (TD). Extruded diets were produced as 3 and 4.5 mm pellets by Biomar (Nersac, France). The MD included 45% of marine origin feedstuffs, containing 30% of fish meal (herring), 10% of anchovy oil and 5% of krill meal, while TD diet included only 25% of these ingredients, 15% of fish meal and 10% of anchovy fish oil, and the 20% less of marine origin

feedstuffs replaced by terrestrial origin feedstuffs, 10% of animal origin, with 5% of spray-dried blood and 5% of feather meal, and 10% of vegetable origin, with 9% of extruded peas seed meal and 1% of vegetable oil (soybean). An economic assessment of the cost of the two diets (€/100 kg) was made considering the price and the percentage of inclusion in the diets of each feedstuffs (Table 1).

Fish were fed twice a day at 8:00 and 17:00, at a rate of 1.7–2% of their body weight, 6 days per week. No fish died during the growth period. At the end of the experiment, 10 fish from each group were killed. Whole muscle fillets were separated and used to determine fatty acid composition and proximate analysis.

Feedstuffs and meat analysis

Moisture, crude protein, lipid and ash of feedstuffs (Table 2) and fillets (Table 3) were determined

Table 1. Ingredients (%) and cost (€/100 kg) of the experimental diets.

	Diets	
	TD	MD
Fish meal (herring)	15	30
Anchovy oil	10	10
Krill meal	–	5
Soybean oil	1	–
Peas meal	9	–
Soybean meal	12	12
Rapeseed meal	10	10
Sunflower meal	10	10
Wheat	5	5
Gluten	11	11
Feather meal	5	–
Spray-dried blood	5	–
Premix ^a	7	7
Cost of the diets (€/100 kg)	82	98

TD: terrestrial diet; MD: marine diet.

^aSupplied the following mg/kg feed (α -tocopherol, 185.0; Thiamin, 12.0; Riboflavin, 20.0; Niacin, 45.0; Pyridoxal phosphate, 11.0; Menadione, 10.0; Biotin, 0.35; Folic acid, 15.0; Ascorbate phosphate, 105.0; Zn, 102.0; I, 1.4; Mg, 30.0; Co, 1.0).

Table 2. Proximate analysis (% dry basis) and selected fatty acid composition (g/100g of total fatty acid methyl esters) of the diets.

	Diets	
	TD	MD
Analysed chemical composition		
Crude protein	39.9	40.1
Crude lipids	14.9	15.0
Ash	7.2	7.6
Main individual methyl esters fatty acids ^a		
C16:0	12.69	15.25
C18:1 n-9	26.95	19.90
C18:2 n-6	11.87	9.05
C18:3 n-3	3.33	1.82
C20:4 n-6	0.65	0.69
C20:5 n-3	3.51	8.44
C22:6 n-3	7.85	10.10

TD: terrestrial diet; MD: marine diet.

^aMain FA selected from a list of identified FA on the chromatograms.

following the methods of Association of Official Analytical Chemists (AOAC 1995). Feed samples were analysed for fatty acid composition using the method of Grey et al. (1967; FA were expressed as percent of total FA methyl esters, FAME; Table 2).

Lipids from tissue were extracted according to the method used by Folch et al. (1957). Duplicates of 100 mg of lipid extracted were methylated by adding 1 mL of hexane and 0.05 mL of 2 N methanolic KOH, and nonanoic acid (C9:0) was used as an internal standard. Gas chromatography analysis was performed on a Varian model CP 3900 instrument equipped with a CP-Sil 88 capillary column (length 100 m, internal diameter 0.25 mm, film thickness 0.25 µm). Operating conditions were a helium flow rate of 0.7 mL/min, an FID detector at 260 °C, a split-splitless injector at 220 °C with an injection rate of 120 mL/min, an injection volume of 1 µL. The temperature of the column was 4 min at 140 °C and a subsequent increase to 220 °C at 4 °C/min. Retention time and area of each peak were computed using the Varian Star 3.4.1. software (Walnut Creek, CA). The individual FA peaks were identified by comparison of retention times with those of known mixtures of standard FAs (FAME mix 37 components from Supelco Inc., Bellefont, PA) run under the same operating conditions. Fatty acids were expressed as percentage of total methylated FA.

Statistical analysis

The data are reported as mean values ± standard error of mean. The significance of differences between the fish from MD and TD groups was assessed using Minitab (Minitab Inc., State College, PA) using the general linear model to perform an analysis of variance. Results were considered as significant at $p < .05$.

Results and discussion

Chemical composition and fatty acid profile of the feeds is presented in Table 2. TD diet exhibited lower level of palmitic acid (C16:0) than those of the MD

Table 3. Proximate composition of the fillets of sea bass fed the experimental diets.

	Dietary treatment		SEM	Significance
	TD	MD		
Number of fish	10	10	10	
Dry matter, %	27.35	28.79	0.536	ns
Ash, %	1.28	1.26	0.104	ns
Lipids, %	3.83	4.41	0.481	ns
Crude protein, %	20.09	19.88	0.300	ns

TD: terrestrial diet; MD: marine diet; ns: not significant; SEM: standard error of means.

diet (12.69 vs 15.25% of total FAME, respectively for TD and MD diets), while the level of oleic acid (C18:1 n-9) was higher in TD diet than in MD diet (26.95% vs 19.90% of total FAME, respectively for TD and MD diets). Also, the levels of linoleic acid (C18:2 n-6) and linolenic acid (C18:3 n-3) were higher in TD diet than in diet included 45% of marine origin feedstuffs, 11.87 vs 9.05 for linoleic acid and 3.33 vs 1.82 for linolenic acid. Consequently, the higher levels of marine origin feedstuffs, the level of the two important HUFA, EPA and DHA, resulted higher in MD diet than in TD diet, 8.44 vs 3.51 for EPA acid and 10.10 vs 7.85 for DHA.

In this trial, the fish responded well to the experimental diets. No mortality and good growth of the groups indicated that all the tested diets did not have adverse effect on the health of the fish. Feeding diets with different fatty acid composition had no effect on weight gain (WG) and feed conversion ratio (FCR) in sea bass (Table 4). The partial replacing of marine origin feedstuffs in the diet (almost 50%) with a mixture of animal and vegetable terrestrial protein sources could be a good tool in the reduction of the production costs. In fact, in this trial the price of TD feed was considerably lower than the price of MD feed, about 82 €/100kg vs 98 €/100kg (Table 1).

Previous studies (Eroldogan et al. 2013) reported that replacement of fish oil with vegetable oils did not negatively impact the growth performance. Similar results were found by Gouveia & Davies (2000) using a diet with a partial substitution of fish meal by pea seed meal in juvenile sea bass. In our study the diet did not affect the total lipid content in fillet. These data, considering that the experimental diets were formulated to be iso-energetic, iso-proteic and with a constant lipid content of about 15%, were predictable.

The proximate composition data expressed in Table 3 revealed no significant differences between each of the nutrient components for sea bass fed the respective experimental diets as observed by Gouveia & Davies (2000) using a diet with a partial substitution of fish meal by pea seed meal in sea bass.

Table 4. Growth parameters of the fish during the experiment.

	Dietary treatment		SEM
	TD	MD	
IBW, g	30.2	30.8	3.6
FBW, g	324.4	332.3	9.9
^a WG, g	294.2	301.5	9.6
^b FCR	1.9	1.9	0.1

There were no significant differences between diets.

TD: terrestrial diet; MD: marine diet; SEM: standard error of the mean; IBW: initial body weight; FBW: final body weight.

^aWG, weight gain = FBW – IBW.

^bFCR, feed conversion ratio = feed consumption/WG.

Table 5. Identified fatty acids of the fillets of sea bass fed the experimental diets (g/100 g of total fatty acid methyl esters).

	Dietary treatment			Significance
	TD	MD	SEM	
Number of fish	10	10	10	
C12:0	0.04	0.04	0.001	ns
C14:0	2.36	3.11	0.124	$p < .05$
C16:0	15.26	15.82	0.189	ns
C16:1 n-7	3.57	4.38	0.142	$p < .05$
C17:0	0.28	0.32	0.008	ns
C17:1	0.44	0.58	0.023	$p < .05$
C18:0	3.52	3.69	0.051	ns
C18:1 n-9	29.63	23.36	1.020	$p < .001$
C18:2 n-6	11.08	9.16	0.313	$p < .001$
C18:3 n-6	0.20	0.20	0.005	ns
C18:3 n-3	2.61	1.61	0.158	$p < .001$
C20:0	0.27	0.25	0.004	ns
C20:1	0.28	0.34	0.010	ns
C20:3 n-6	2.07	2.11	0.026	ns
C20:3 n-3	0.58	0.61	0.013	ns
C20:4 n-6	0.69	0.79	0.023	$p < .05$
C22:0	0.37	0.47	0.016	ns
C20:5 n-3	4.14	6.39	0.359	$p < .001$
C20:1 n-9	0.90	1.13	0.038	ns
C22:5 n-3	1.08	1.42	0.058	$p < .001$
C22:6 n-3	8.95	9.72	0.169	$p < .01$
Unknown	11.49	14.52	0.706	ns
SFA	22.37	24.04	0.329	$p < .01$
MUFA	37.21	32.31	0.836	$p < .001$
PUFA	31.40	32.01	0.242	ns
$\sum n-3$	17.36	19.75	0.407	$p < .001$
$\sum n-6$	14.04	12.26	0.300	$p < .001$
$\sum n-6/\sum n-3$	0.81	0.62	0.030	$p < .01$

TD: terrestrial diet; MD: marine diet; ns: not significant; SEM: standard error of means; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Although the percentage of fish oil, the main lipid source, was the same in both diets (10%), inclusion of animal and vegetable origin feedstuffs in TD diet, in place of marine origin feedstuffs, modified fillet fatty acid profile (Table 5). Often in marine fish species with limited action to convert C18 fatty acids this effect is more evident.

The level of saturated fatty acids (SFA) was lower ($p < .01$) in fillets from fish fed with the diet containing higher percentage of terrestrial origin feedstuffs compared to the level found in fillets from fish fed with the MD diet. In contrast, C18:1 n-9 content increased in the muscle of fish fed the diet with higher levels of terrestrial origin feedstuffs, proportionally its increase in the diet. Similar results were observed by Xue et al. (2006) using animal and vegetable alternative lipid sources. Consequently, the higher percentage of this fatty acid, the most present in the diets, the level of MUFA was significantly higher ($p < .001$) in fillets from fish fed with TD diet than in fillets from fish fed with MD diet.

In the diet, the inclusion of animal and vegetable origin feedstuffs in place of marine origin feedstuffs reduced the level of EPA and DHA, despite the percentage of fish oil, the main lipid source, was the

same in both diets. This data is probably also influenced by the use of krill meal in MD diet, an ingredient particularly rich in n-3 HUFA (Tandy et al. 2009). Consequently, feeding with a diet richer in terrestrial origin feedstuffs reduced fillet content of EPA and DHA. However, it is interesting to note that the reduction of EPA and DHA level in sea bass from TD group was to a lower extent than could be expected by their reduction in the diets consequently to the partial replacement of marine origin feedstuffs by terrestrial origin feedstuffs. In fact, while in MD diet compared to TD diet the level of EPA and DHA resulted higher 2.40 and 1.29 times, respectively, in fillets from fish of MD group the level of these two n-3 HUFA resulted higher just 1.54 and 1.08 times respectively for EPA and DHA. Turchini and Francis (2009) observed lower β -oxidation of n-3 HUFA, and therefore higher deposition, in tissues of Murray cod (*Maccullochella peelii peelii*) fed with abundant MUFA and to a lesser extent SFA.

As showed in other studies (Xue et al. 2006), concentrations of these important n-3 HUFA in fish fed alternative lipid sources were usually lower in fillets from fish fed with diets enriched with vegetable origin feedstuffs in place of marine origin feedstuffs. Some animal and vegetable origin feedstuffs used for the production of fish diets don't provide high levels of n-3 HUFA and their higher inclusion in TD diet, at the expense of marine origin feedstuffs, consequently reduced the levels of n-3 fatty acids (Table 2). It is well-known that marine fish require mainly n-3 HUFA and, in particular, C20:5 n-3 and C22:6 n-3 for optimum performance (Eroldogan et al. 2013). Addition to being considered indispensable for marine species, the n-3 HUFA showed a wide range of biological effects which are believed to be beneficial for human health (Siddiqui et al. 2008). Therefore, today is considered a very important factor to increase their presence in products for human consumption and consequently reduction of n-3 HUFA caused by marine origin feedstuffs substitution may negatively affect the fillet nutritional value and fish welfare. However, as reported previously, the reduction of EPA and DHA level in sea bass fillets from fish of TD group was to a lower extent than could be expected by their reduction in the diets.

The main representative of n-6 fatty acids in dietary lipids is linoleic acid. This is accumulated largely unchanged in the lipids of marine fish due to their reduced capacity for chain elongation and desaturation (Parpoura & Alexis 2001). Compared to the fish fed with TD diet, fish from MD group exhibited a lower concentration of linoleic acid ($p < .001$) and a lower level of the ratio $\sum n-6/\sum n-3$ ($p < .01$). However, the level of the ratio $\sum n-6/\sum n-3$ in fillet from fish of

TD group maintains a value acceptable, considering the values recommended by Brennal et al. (2009). Also, the level of C18:3 n-3 was higher ($p < .001$) in fish muscle fed with TD diet than in fish muscle fed with MD diet. These data were closely linked to the higher percentages of these two essential fatty acids in the diet in which marine origin feedstuffs were replaced by terrestrial origin feedstuffs.

Despite the higher content of linoleic acid in the TD diet, the level of C20:4 n-6 was lower ($p < .05$) in fillets from fish fed with this diet than in fillets from fish fed with the MD diet, suggesting a possible *in vivo* fatty acid conversion, as also found by Eroldogan et al. (2013) who observed a reduction of C20:4 n-6 in European sea bass muscle with the inclusion of vegetable oils in the diet.

Conclusions

In conclusion, the results of this study show that the inclusion of a mixture of terrestrial origin feedstuffs in place of marine origin feedstuffs did not affect the growth performance and food conversion ratio, an important result considering that the price of TD feed was considerably lower compared with the price of MD feed. However, in this trial the inclusion of a mixture of animal and vegetable terrestrial origin feedstuffs reduced the levels of total n-3 PUFA, especially of EPA and DHA, although the reduction of the levels of these two important n-3 HUFA in fillets from sea bass of TD group was to a lower extent than could be expected by their levels in TD diet in confront of MD diet. According to the effect of treatments on total n-3 PUFA, the value of $\sum n-6 / \sum n-3$ ratio was higher in fillets of sea bass fed TD diet, maintaining anyway the values sufficiently acceptable.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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