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in

Tacon A.G.J. (ed.), Basurco B. (ed.).
Feeding tomorrow's fish

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 22

1997
pages 183-204

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=97605921>

To cite this article / Pour citer cet article

Alexis M.N. **Fish meal and fish oil replacers in Mediterranean marine fish diets.** In : Tacon A.G.J. (ed.), Basurco B. (ed.). *Feeding tomorrow's fish*. Zaragoza : CIHEAM, 1997. p. 183-204 (Cahiers Options Méditerranéennes; n. 22)



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Fish meal and fish oil replacers in Mediterranean marine fish diets

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SUMMARY - A number of experiments were performed in order to evaluate different feedstuffs as fish meal and fish oil replacers in Mediterranean marine fish diets, including the European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). Apparent crude protein digestibilities were relatively high for most feedstuffs, while energy digestibilities varied over a great range. Selected feedstuffs were further tested concerning their potential to replace white fish meal protein in gilthead bream diets. Meat and bone meals performed well up to 40% substitution of fish meal protein. Good quality poultry meal could replace 100% of white fish meal without a significant loss in fish performance. However, feather meal could be tolerated at lower levels. Different soya products were also tested. Good results were obtained using adequately heated full fat soya substituting 35% of fish meal protein while soybean meal could be tolerated at lower levels. A soya protein concentrate, tested at 35% inclusion level, provided significantly lower growth. Plant oils do not cover EFA requirements of Mediterranean fish and result in severe histological lesions if used singly as an oil supplement. Adequate amounts of n-3 PUFA, supplied by fish oils, are required to suppress histological lesions. Tissue lipids reflect dietary lipid composition and could possibly contribute to the taste of fish. Comparison of tissue fatty acid composition of wild and cultured sea bream and sea bass revealed certain differences, wild fish containing higher levels of arachidonic acid than cultured fish. In view of the importance of arachidonic acid for various physiological functions adequate enrichment of diets might be necessary for optimum fish performance.

Key words: *Sparus aurata*, *Dicentrarchus labrax*, digestibility, fish meal, soya, poultry by products, feather meal, meat and bone meal, fish oil, fatty acids.

RESUME - "Produits de remplacement des farines et huiles de poisson pour les régimes des poissons méditerranéens." Une série d'expériences a été effectuée afin d'évaluer différents aliments en vue de remplacer la farine et l'huile de poisson dans les régimes alimentaires des poissons méditerranéens, parmi lesquels le bar européen (*Dicentrarchus labrax*) et la daurade (*Sparus aurata*). La plupart des aliments testés ont montré une digestibilité apparente de la protéine brute assez élevée, tandis que l'énergie digestible a varié sur une grande échelle. Les aliments sélectionnés ont été en outre analysés concernant leur potentiel de remplacement des farines de poisson comme source de protéines dans l'alimentation de la daurade. La substitution par des farines de bétail et d'os, jusqu'à 40% de la farine de poisson a donné de bons résultats. La farine de volaille de bonne qualité pourrait remplacer à 100% la farine de poisson sans aucune perte significative au niveau des performances des poissons. Par contre la farine de plume pourrait être tolérée à un niveau inférieur. Différents produits à base de soja ont été également testés. La substitution de 35% de la farine de poisson par du soja non dégraissé et chauffé à la température adéquate, a donné de bons résultats. La farine de grains de soja pourrait être tolérée à un taux d'incorporation inférieur. L'utilisation d'un concentré protéique de soja, testé à 35% de taux d'incorporation, a conduit à une croissance significativement réduite. Les huiles végétales ne couvrent pas les besoins en acides gras essentiels des poissons méditerranéens et dans le cas d'une substitution totale, conduisent à de sévères lésions histologiques. Des quantités suffisantes en acides gras polyinsaturés n-3 assurées par les huiles de poisson, sont nécessaires pour supprimer les lésions histologiques. Les lipides tissulaires reflètent la composition lipidique du régime alimentaire et pourraient contribuer au goût du poisson. Une comparaison de la composition des acides gras tissulaires entre les daurades et les loups du milieu naturel et ceux d'élevage a mis en évidence des différences, les poissons sauvages contenant des niveaux supérieurs d'acide arachidonique par rapport aux poissons d'élevage. En vue de l'importance

de l'acide arachidonique pour plusieurs fonctions physiologiques, un enrichissement adéquat des régimes alimentaires permettrait d'obtenir des performances optimum chez les poissons.

Mots-clés : Sparus aurata, Dicentrarchus labrax, digestibilité, farine de poisson, soja, farine de bétail, farine de volaille, farine de plume, huile de poisson, acides gras.

INTRODUCTION

Fish meal is the main dietary protein source in aquaculture feeds (Tacon and Jackson, 1985, Kaushik, 1989). Fish oil is also used as the main oil supplement in fish diets in order to increase their energy content and provide essential fatty acids. The supply of these materials is limited and their cost is continuously increasing affecting in a direct way feeding costs and total production costs in aquaculture. For this reason considerable research efforts have been directed towards the evaluation of other ingredients as potential substitutes in fish diets (Tacon and Jackson, 1985).

Sea bream and sea bass are the major marine fish cultured in Mediterranean countries. Intensive aquaculture of these species in Greece has greatly expanded during last years (Stefanis, 1995). However there is a lack of information concerning the potential of including different feedstuffs in practical diets for these species.

The present paper reviews of the research conducted during recent years concerning the evaluation of the nutritional value of different raw materials as feed ingredients for these Mediterranean marine fish species as well as the optimum levels of inclusion of fish oils in their diets.

MATERIALS AND METHODS

Diet preparation

All diets for gilthead bream were prepared in the laboratory after blending dry dietary ingredients thoroughly in a Hobart bench food mixer. The required amount of oil and an appropriate amount of water to form a soft dough were added into the dry mixture. Diets were cold pelleted and dried in a convection dryer either at 40°C (experiments for testing fish meal replacers) or at ambient temperature (experiments for testing fish oil replacers).

The diets for sea bass were prepared by a local factory using common compression procedures and supplemental oil was added on the pellets by spraying.

Fish meal replacers

Digestibility studies

These studies were performed using sea bream and sea bass having an average initial weight of 45g and 25g respectively, in specially designed cylindroconical

tanks. A mixture of 40% herring meal and 60% wheat middlings served as the fixed component of the basal test diets at an inclusion level of about 50%. The remainder was contributed by the different test ingredients (Nengas et al., 1995). Digestibility was measured by the use of an inert indicator (Cr_2O_3) incorporated in the diet at 1% level.

Substitution of fish meal protein using soya products and by-products

Solvent extracted soybean meal (SES) was first tested at different levels of substitution of white fish meal (WFM) protein. The basic composition of the diets used is given in Table 1. The initial weight of fish was 6.2g and fish were fed at a constant rate of 3% of their body weight/day at a temperature of 22° C (Nengas et al., 1996).

Table 1. Basic composition of diets used for testing solvent extracted soya bean substitution (SES) of fish meal protein*

	FM	SES 10	SES 20	SES 30	SES 40
<i>Formulation</i>					
White fishmeal	74.0	66.6	59.2	51.8	44.4
SES	-	11.9	23.7	35.6	47.5
Cod Liver Oil	3.6	4.0	4.5	4.9	5.3
Starch/Dextrin	14.0	0.6	7.1	3.7	0.2
á-Cellulose	6.4	4.9	3.5	2.0	-
<i>Nutrient analysis (as fed basis)</i>					
Protein	47.5	47.7	47.7	48.0	48.1
Lipid	9.6	9.5	9.6	9.7	10.0
Ash	16.1	15.4	14.7	14.5	14.0
NFE	14.1	15.5	15.6	14.8	14.6
Energy (Mj/Kg)	17.6	17.8	18.4	18.3	18.6

* Origin of materials tested, Nengas et al., (1996)

Different soybean products were tested in the next experiment at a constant level (35%) of substitution of WFM protein. The basic composition of diets used is given in Table 2. The initial weight of fish was 1.6g, water temperature 22°C, and feeding level fixed at 4% of fish body weight. The raw materials used were tested for their trypsin inhibitor (TI) levels, cresol red values and available lysine as described by Nengas et al., (1996).

Substitution of fish meal using poultry by-products

Poultry by-products of different origin were tested in two experiments. In the first experiment low levels of dietary inclusion of a high fat poultry meal (PM), before and after defatting (DPM), and a high quality poultry meal (PMM) were evaluated (Table 3). The experimental fish employed had an initial weight of 1.1g, fed at a fixed level of 5% body weight per day, and water temperature maintained at 22°C.

During the second experiment high levels of PMM were tested as well as mixtures with feather meal (FM) at a 3:1 ratio and two different local products, PBP1 and

PBP2, differing in their fat content (Table 4). The fish employed had an average initial weight of 1.5g, fed at fixed rate of 4% body weight/day. Water temperature was maintained at 22°C.

Table 2. Basic composition of diets used for evaluating nutritional value of different soybean products.

	FM	LS	SS	HS	SES	DAN
<i>Formulation</i>						
White fishmeal	71.9	46.7	46.7	46.7	46.7	46.7
LS	-	42.5	-	-	-	-
SS	-	-	45.7	-	-	-
HS	-	-	-	44.3	-	-
SES	-	-	-	-	36.6	-
DAN	-	-	-	-	-	23.6
Cod Liver Oil	6.97	0.16	-	-	8.07	8.61
Starch/Dextrin	10.0	1.02	2.04	0.70	-	5.94
a-Cellulose	8.03	4.01	-	2.74	3.07	9.54
<i>Nutrient analysis (as fed)</i>						
Protein	44.1	45.0	46.9	45.2	45.1	45.2
Lipid	12.8	12.1	12.7	12.6	12.5	12.4
Ash	15.2	14.1	13.1	12.2	12.0	10.9
NFE	13.2	13.2	16.5	16.7	16.7	13.6
Energy (Mj/Kg)	17.9	18.3	17.6	17.8	17.8	17.9

LS, SS, HS: full fat soya heated at 158°C and cooked at 110°C for 5, 20 and 45 min respectively. SES: solvent extracted soya, DAN: Danpro A, a soya protein concentrate, Nengas et al., (1996)

Table 3 Basic composition of diets containing low levels of different poultry by-products.

	FM 100%	PM 20%	DPM 35%	PMM 20%	PMM 35%	PMM 50%
<i>Formulation</i>						
White fishmeal	74.0	59.2	48.1	59.2	48.1	37.0
PMM	-	-	-	15.8	27.6	39.5
PM	-	18.3	-	-	-	-
DPM	-	-	25.2	-	-	-
Cod Liver Oil	5.60	1.38	5.77	4.76	4.12	3.48
Starch/Dextrin	12.0	12.0	12.0	12.0	12.0	12.0
á-Cellulose	6.2	6.97	6.75	6.06	5.96	5.87
<i>Nutrient analysis</i>						
Protein	47.1	47.2	48.3	48.8	48.6	48.0
Lipid	9.3	9.7	9.18	9.73	9.67	9.14
Ash	16.4	15.7	12.8	15.9	15.9	15.3
NFE	14.3	14.2	15.7	11.8	12.3	12.0
Energy (Mj/Kg)	18.2	18.4	18.7	17.7	17.5	17.0

PM: high fat poultry meal, DPM: PM after deffating, PMM: high quality poultry meat meal

Substitution of fish meal by meat and bone meal

A conventional meat and bone meal (MB) containing about 60% protein, 9% fat and 25% ash was used substituting 20 and 40% of fish meal protein (Table 5). Fish of mean initial weight of 5g were used and fed at a fixed level of 3% body weight/day. Water temperature was maintained at 22° C.

Table 4. Basic composition of diets containing high levels of poultry by-products.

	FM	PMM 75	PMM 100	PMM/ FM75	PMM/ FM100	PB1 40	PBP2 35	PBP2 50	PBP2 75
<i>Formulation</i>									
White fish meal	72.9	18.8	-	18,0	-	42.1	45.7	35.2	18.0
PMM	-	53.6	71.4	-	-	-	-	-	-
PMM/FM	-	-	-	50.3	67.0	-	-	-	-
PBP1	-	-	-	-	-	-	20.4	29.2	43.8
PBP2	-	-	-	-	-	29.5	-	-	-
Cod Liver Oil	6.97	5.12	4.50	5.97	5.64	1.12	5.75	5.58	5.28
Starch/Dextrin	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
á-Cellulose	3.53	3.55	3.56	4.56	4.89	6.58	7.51	9.47	12.74
<i>Nutrient analysis</i>									
Protein	44.5	44.7	44.9	44.7	44.5	44.9	45.6	46.2	44.9
Lipid	12.5	12.6	12.4	12.0	14.1	14.4	12.6	13.1	12.5
Ash	15.3	14.1	13.5	12.8	12.4	12.1	12.0	10.5	7.20
NFE	16.1	17.9	18.6	19.6	17.8	14.1	15.5	13.9	17.2
Energy (Mj/Kg)	17.6	18.1	18.2	18.1	18.0	18.6	18.5	19.1	18.4

PMM: poultry meat meal, FM: feather meal, PBP1: high fat local product, PBP2: lower fat local product

Table 5. Basic composition of diets containing different levels of meat and bone meal.

	FM	MB 20	MB 40
White fish meal	74.0	62.3	46.7
MB	-	16.5	32,9
Cod Liver Oil	6.97	5.12	4.50
Starch/Dextrin	8.0	8.0	8.0
á-Cellulose	12.3	7.9	7.2
<i>Nutrient analysis</i>			
Protein	47.2	49.7	49,2
Lipid	9.2	9.4	9,2
Ash	17.0	18.2	19.2
NFE	7.2	8.8	9.7
Energy (Mj/Kg)	18.9	19.2	19.4

Fish oil replacers

Sea bream

In the first experiment different plant oils (SBO:soybean oil, OO:olive oil) or mixtures of olive oil with different fish oils (CLO:cod liver oil, BO:boost oil, FO:commercial fish oil) were used as supplements within purified diets. A fat free diet (FF) was also used. The composition of the main fatty acids within the dietary lipids tested is shown in Table 6. Experimental gilthead bream had an average initial weight of 1.2g, and were fed at a fixed level of 5 to 4% body weight/day, and water temperature maintained at 23° C.

Table 6. Fatty acid composition (% of total fatty acids) of experimental diets containing different plant and fish oil mixtures.

	O.O	FF	SBO	OO/CLO	OO/BO	D.O
Saturated.	18.64	32.30	16.70	18.73	21.56	23.10
Sum n-9	68.08	16.90	3.75	57.62	53.95	17.34
Sum n-6	8.39	12.85	49.91	7.23	7.70	4.24
Sum n-3	1.02	4.81	7.83	4.98	7.81	28.04
EPA+DHA	0.28	3.88	0.28	3.61	5.48	20.59

In a second experiment soybean oil was tested at different levels of substitution of cod liver oil. A purified diet supplemented with 12% total lipid was used. Dietary CLO increased in consecutive intervals of two units while the level of SBO was respectively reduced. The dietary composition of the main fatty acids of the diets fed is given in Table 7. The initial mean weight of fish was 1.2g, and fish fed at a fixed level 6-5% body weight per day. Water temperature was maintained at 20°C (Kalogeropoulos et al., 1992).

Table 7. Fatty acid composition (% of total fatty acids) of the experimental gilthead bream diets containing reciprocating amounts of cod liver oil (CLO) and soybean oil (SBO).

		Experimental diets					
	% CLO	2	4	6	8	10	12
Saturates		15.9	16.3	16.7	17.2	17.6	18.0
Monoenes		11.0	18.5	26.5	34.5	42.0	50.1
SUM n-9		6.8	10.7	14.8	19.0	22.9	27.0
SUM n-6		44.4	36.4	27.9	19.5	11.5	3.0
SUM n-3		9.7	11.3	13.0	14.6	16.2	17.8
EPA+DHA		2.4	4.7	7.2	9.6	11.9	14.4
EPA+DHA	% of diet	0.30	0.60	0.9	1.2	1.5	1.9

Sea bass

Practical type diets were employed during this experiment containing a considerable quantity of fish meal and therefore of fish oil (3.5% of the diet). The diets were supplemented with about 5.5% oil which was either soybean or olive oil or mixtures of the previous oils with fish oil or pure fish oil. The fatty acid composition of the diets is given in Table 8. The mean initial size of fish was 50g, and fish fed at a fixed level of 2-1.7%, and water temperature ranged between 18-25.5°C.

Table 8. Fatty acid composition (% of total fatty acids) of the experimental sea bass diets containing plant oils or mixtures of plant oils with fish oils.

	O.O	SBO	SBO/ FO	OO/FO	FO
Saturates	23.3	22.6	23.5	23.9	22.9
Monoenes	51.2	26.5	35.0	46.2	38.0
Sum n-6	10.2	33.0	20.5	10.2	9.8
Sum n-3	9.1	11.7	13.6	12.5	14.2
EPA+DHA	6.9	6.9	9.2	9.2	10.5

Comparison of wild and cultured fish

The fatty acid composition of tissues of sea bream and sea bass caught from the wild, either from Messolonghi lagoon (M) or from Evoikos gulf (E) were compared with cultured fish. Comparison was made at the same time of the year, at the beginning of winter, and for similar fish weights (100-150g).

RESULTS AND DISCUSSION**Fish meal replacers***Digestibility studies*

Apparent crude protein digestibility coefficients (ADPC) varied among feedstuffs tested (Table 9), although most feedstuffs indicated high coefficients for both fish species. The highest digestibility coefficients were recorded for herring meal and skimmed milk powder with gluten meal and soybean meal displaying only slightly inferior digestibility. All other ingredients performed well with values greater than 60%; the only exception being, feather meal A and blood meal in the case of gilthead bream.

Energy digestibilities varied considerably, with animal by products generally having higher digestibilities than plant feedstuffs. As before, the bulk of the digestibility value for the animal by-products were higher than 60%, with herring meal and skimmed milk displaying the highest digestibility coefficients. Of the plant

materials tested corn gluten meal and full fat soya displayed the highest digestibility values.

It is well known that the digestibility of feedstuffs depend upon a variety of different factors, including the type and extent of processing technology applied during the preparation of the feedstuff and/or manufacture of the finished feed. For example excessive heat treatment of animal materials during the drying process can severely reduce their protein digestibility; the protein digestibility of blood meal varying from as low as 16% within flame dried bloodmeal to as high as 99% within spray dried meals (Cho et al., 1982). The value obtained during the present study (46) was an intermediate value between those reported by Cho et al. (1982), and possibly the result of the less severe steam drying process employed for the preparation of this material.

Table 9. Apparent digestibility coefficients (%) of common feed ingredients tested for sea bass and sea bream *.

	Sea bream		Sea bass
	Protein	Energy	Protein
<i>Animal by-products</i>			
Herring meal (68/12)**	95,8	94,1	96,0
Poultry by-product meal (51/29)	81,8	80,3	84,5
Poultry meat meal (65/14)	89,9	67,4	-
Feather meal (A) (82/2)	24,9	6,7	-
Feather meal (B) (81/6)	57,5	63,9	81,5
Meat and bone meal (59/9)	72,2	69,2	92,2
Skimmed milk (32/0.4)	95,5	104,3	-
Blood meal (92/0.5)	46,3	57,8	-
<i>Plant by-products</i>			
Soybean meal (44/1)	90,9	44,7	88,4
Full fat soya meal (34/23)	75,7	61,9	-
Corn gluten meal (66/5)	90	79,7	93,8
Corn gluten feed (19/7)	65,3	23,7	-
Flaked maize (8/4)	60,3	33,7	-
Sunflower meal (32/1)	86,2	-36,0	91,3
Cottonseed meal (42/4)	75,4	39,2	87
Tomato pulp meal (21/8)	20,1	8,8	-

(*)Details about the origin of raw materials are given in Nengas et al., (1995)

(**) Crude protein %/Ether extract %

Differences in digestibilities were also noted for the two feather meals tested. Feather meal contains keratin which is not easily digested by fish unless proper processing conditions of heat and pressure are applied. For example product A was a local product and the treatment followed for its production does not appear to have been adequate for obtaining high digestibility values.

The generally lower energy digestibilities of plant by products tested can largely be attributed to their higher crude fibre and carbohydrate content; crude fibre generally being considered indigestible for most carnivorous fish species

(Kirchgessner et.al. 1986; Bergot 1981). Furthermore the existence of high levels of fibre may also interfere with the digestion of other nutrients (Anderson 1985; Buddington and Hilton 1987) and therefore reducing the overall digestibility of the diet. The highest level of crude fibre was found in tomato pomace, corn gluten feed, sunflower meal and flaked maize ; these materials also displaying the lowest energy digestibility values among the different plant materials tested. For example, Davies (1985) reported negative digestibility coefficients for sunflower meal with seabream; the erroneous results are largely believed to have been due to the high fibre content of these feedstuffs.

Preliminary data indicated relatively high digestibility for corn starch by gilthead bream (Georgopoulos 1990). However, starch digestibility has been shown to vary greatly in rainbow trout depending upon its botanical origin (Bergot 1993), and this may be one of the major factors affecting the digestibility of the different plant feedstuffs. However since starch digestibility improves with heat treatment (Bergot 1993), it follows therefore that further heat treatment of plant materials exhibiting good protein digestibility may further improve their nutritional value.

Substitution of fish meal with soya products and by-products

Substitution of white fish meal protein with SES protein resulted in reduced fish performance (Figure 1); significant differences appearing after a 30% substitution of WFM protein for weight increase and percent weight gain, and after a 40% substitution for feed efficiency and apparent crude protein utilisation.

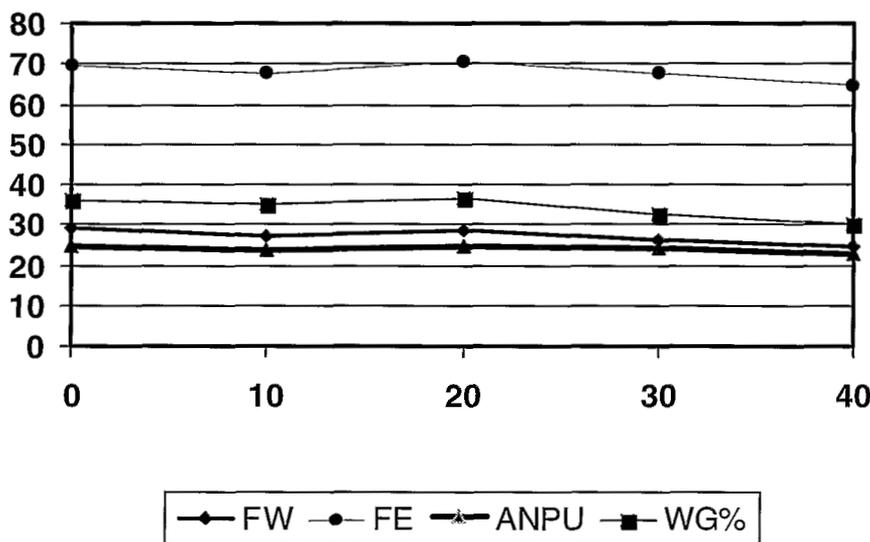


Fig. 1. Performance factors of fish fed diets containing different levels of substitution of white fish meal (WFM) protein with solvent extracted soybean meal. FW: final weight of fish in g, FE: feed efficiency, ANPU: apparent net protein utilisation., WG: % weight gain of fish/10.

Fish fed diets containing SS and HS exhibited similar performance to the fish meal based control diet, while performance declined when LS, SES or DAN were incorporated into the diet (Figure 2). A comparison of the TI activities of the different

heat processed full fat soya is shown in Table 10 and indicated that 67 to 85% of the TI activity was destroyed. Since the HS product displayed the best performance it appears that at least a 85% destruction of TI activity is required for optimum performance of gilthead bream. This value is within the range reported for other species (Sandholm et al., 1976, Viola et al., 1983, Wilson and Poe, 1985).

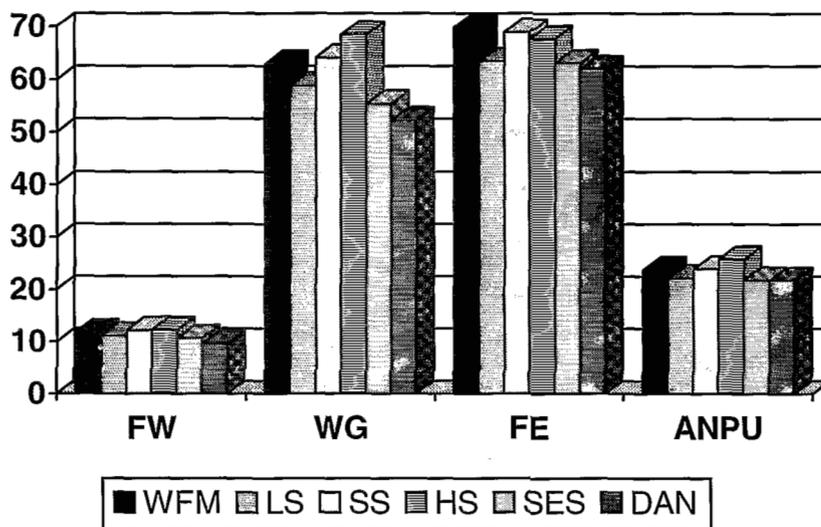


Fig. 2. Performance factors of fish fed diets containing a 35% substitution of white fish meal protein by the protein of different soya products. WFM, white fish meal diet, LS, SS, HS low, standard and high heat full fat soya respectively, SES solvent extracted soya, DAN, danpro soya protein concentrate. FW: final weight of fish in g, WG: % weight gain of fish/10, FE: feed efficiency, ANPU: apparent net protein utilisation.

Table 10. Trypsin Inhibitor Activity (TIA), Cresol red values and available lysine content of the different soybean meals tested

	TIA ¹	Cresol red ²	Available Lysine ³
LS	6.92	3.43	6.52
SS	4.39	3.66	6.51
HS	3.10	3.96	6.21
SES	3.49	3.66	6.46
DAN	3.05	4.73	5.56

1)TIA: mg trypsin inhibited/g meal,
 2) Cresol red expressed as mg dye/g meal,
 3) Available lysine expressed as % of protein

The cresol red binding value is a measure of the degree of denaturation of soya protein and therefore of its digestibility (Olomucki and Bornstein, 1960). The values obtained for the soybean products used during the present study indicate a properly heated product for HS and a slightly underheated product for SS and LS. The value for SES indicated a slightly underheated product whilst the high value obtained for DAN might have been due to its high protein content. From the studies of Kakade et

al. (1973) with rats it appears that about 40% of the pancreatic hypertrophic effect could be accounted for by TI inhibitor activity. The remaining 60% effect was believed to be caused by the resistance of the native protein to the attack by digestive enzymes. The reduced performance of SES within both experiments might therefore have been due to inadequate heat treatment.

Surprisingly, despite the fact that DAN had the lowest TIA and highest cresol red value, it produced inferior growth in fish. Although this may have been due to its lower available lysine content (Table 10), contradictory results have also been shown in the literature regarding the use of this product (Viola, 1983, Davies et al., 1989). These differences have been attributed to the different lysine requirements of the species tested.

Substitution of fish meal with poultry by-products

Substitution of WFM protein with PMM protein resulted in a significant increase in the final mean weight of fish (Figure 3), with PM and DPM products giving values close to the control WFM diet. The same trend was apparent in the other performance parameters indicating that PMM was a good product for inclusion in gilthead bream diets up to the highest level tested of 39.5% (Table 3). Higher dietary inclusion levels of this product during the second experiment (Figure 4 and 5) supported this conclusion, since 75% and 100% substitution of WFM protein resulted in performance values which were only slightly lower than those of the control diet (but not statistically different). However the inclusion of feather meal with PMM in the diets reduced final weights and percent weight gain of fish when 100% of the WFM protein was replaced.

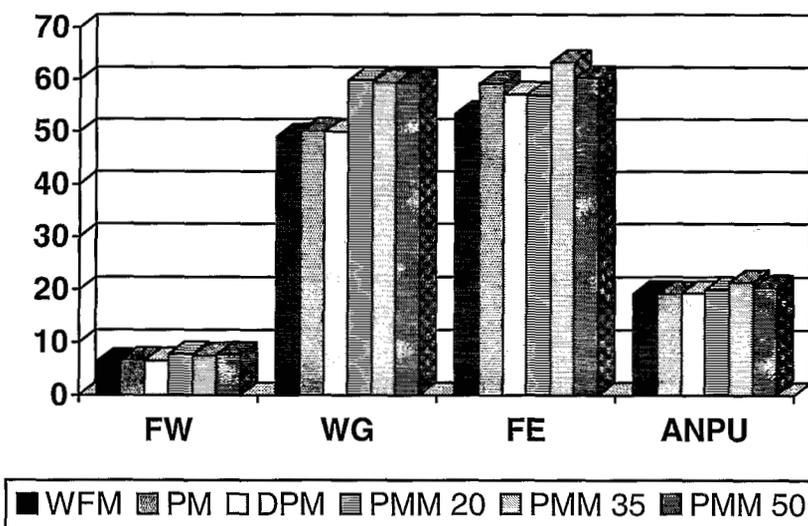


Fig. 3. Performance factors of fish fed diets containing different levels of substitution of fish meal protein (WFM) with poultry meal. PM : high fat poultry meal, DPM: defatted PM, PMM: high quality poultry meal. FW: final weight of fish in g, WG: percent weight gain of fish/10, FE: feed efficiency, ANPU: apparent net protein utilisation.

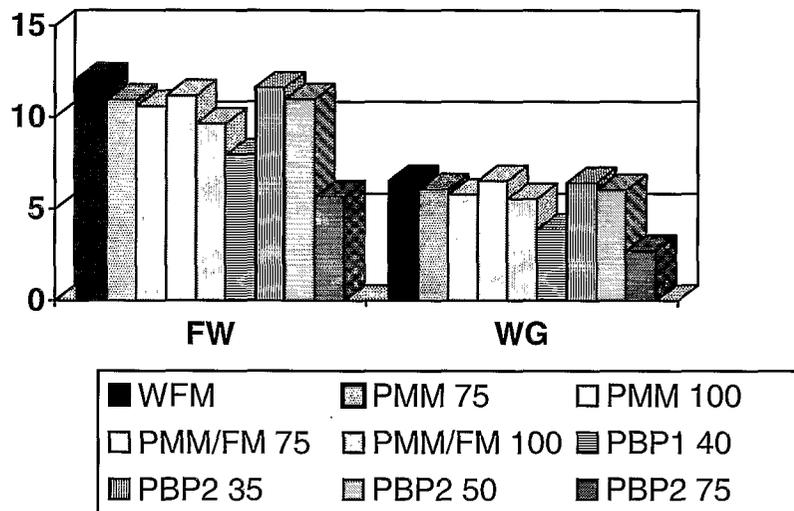


Fig. 4. Final weights (FW in g) and percent weight gain (WG/10) of fish fed diets containing high inclusion of different poultry by-products in substitution of WFM protein. WFM: white fish meal diet, PMM: poultry meat meal, PMM/FM: 3/1 mixture of PMM and feather meal, PBP1: local poultry by-products with a high fat content, PBP2: local poultry by-products with a lower fat content.

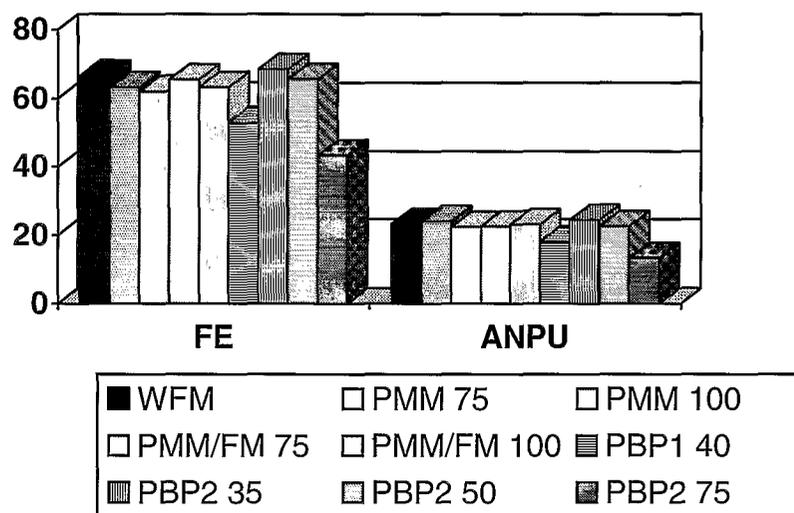


Fig. 5. Feed efficiency (FE) and apparent net protein utilisation (ANPU) of fish fed diets containing high inclusion of different poultry by-products in substitution of WFM protein. WFM: white fish meal diet, PMM: poultry meat meal, PMM/FM: 3/1 mixture of PMM and feather meal, PBP1: local poultry by-products with a high fat content, PBP2: local poultry by-products with a lower fat content.

Locally produced poultry meals were of lower nutritional value than PMM. The local high fat products resulting in lower fish performance, with a maximum 50% inclusion level for the local low fat product without significantly reducing fish performance.

The potential of poultry by-products as fish meal replacers has been indicated in a number of studies with rainbow trout (Tiews et al., 1976, Gropp et al., 1979, Alexis et al., 1985, Steffens, 1985,1987). Within many of these studies a 100% substitution of fish meal protein with poultry by products was possible without any significant reduction in performance parameters. By contrast feather meal is generally considered to be an inferior source of protein for fish because of its poor digestibility and essential amino acid profile. However, Koops et al. (1982) successfully formulated diets containing 14-15% feather meal for rainbow trout (*Oncorhynchus mykiss*) and Tiews et al. (1979) successfully replaced 50% of the protein with feather meal in rainbow trout diets. Finally Fowler (1990) tested levels of up to 15% dietary protein with feather meal and observed no adverse effect on the growth and feed utilisation of chinook salmon (*Oncorhynchus tshawytscha.*). From the results of the present experiment it appears that the capacity of sea bream to utilise feather meal is lower than that of rainbow trout. However it should also be mentioned that the local products used were composed of a mixture of poultry by-products and feathers processed together. This could have been one of the factors resulting in their lower nutritional value.

Substitution of fish meal with meat and bone meal

The maximum level of meat and bone meal inclusion within the gilthead bream diets used in this study was determined by its ash content, with higher ash levels possibly reducing fish performance because of the lower energy content of the high meat and bone meal containing diets. However, the product tested performed well up to its highest inclusion level (Figure 6) and so indicating that it is a promising raw material for substituting fish meal protein. Despite this, the high ash content of meat and bone meals puts certain limitations on its possible use within fish feeds (Hardy, 1989).

Fish oil replacers

Sea bream

The results of both experiments indicated that polyunsaturated fatty acids of the n-3 series were indispensable for obtaining a good performance of gilthead bream (Figures, 7,8 and 9). The utilisation of plant oils as the only source of dietary lipids significantly reduced fish performance. Similar results were also obtained with diets containing low levels of fish oil. The minimum requirement of gilthead bream for n-3 highly unsaturated fatty acids (HUFA) appears to be at least 7% of the dietary fatty acids or about 0.9% of the diet (Figure 9; Kalogeropoulos et al., 1992).

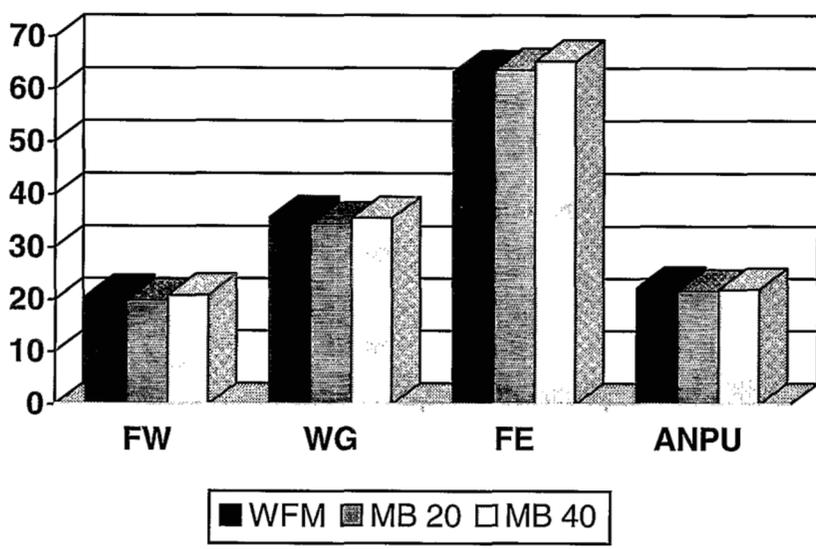


Fig. 6. Performance factors of fish fed diets containing different levels of substitution of white fish meal protein with meat and bone meal. FW: final fish weight in g, WG: % weight gain of fish/10, FE: % feed efficiency, ANPU: apparent net protein utilisation.

The requirements of different fish species for n-3 HUFA have been shown to vary considerably; rainbow trout requiring about 0.5% of the diet as EPA and DHA when these are supplied in equal amounts and channel catfish requiring 0.5 - 0.75% of n-3 HUFA for best performance (Takeuchi and Watanabe, 1977, Satoh et al., 1989), turbot requiring 0.57-0.8% of the diet

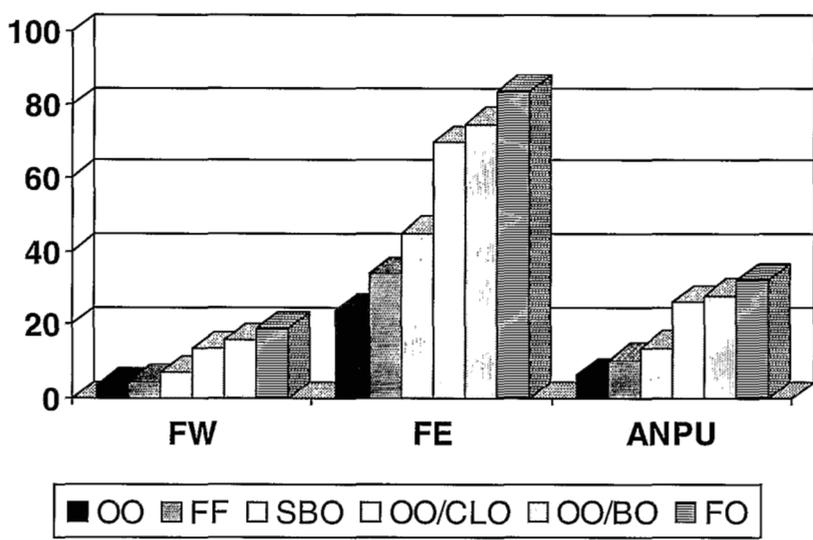


Fig. 7. Performance factors of fish fed diets containing different plant oils or mixtures of olive oil with different fish oils. OO: olive oil, FF: fat free diet, SBO: soybean oil, CLO: cod liver oil, BO: boost oil, FO fish oil. FW: final fish weight in g, FE: % feed efficiency, ANPU: apparent net protein utilisation.

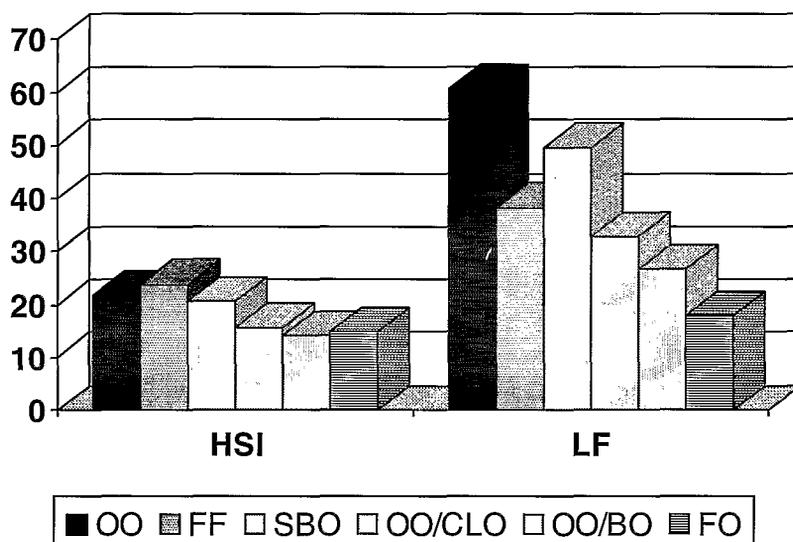


Fig. 8. Liver characteristics of fish fed diets containing different plant oils or mixtures of olive oil with different fish oils. OO: olive oil, FF: fat free diet, SBO: soybean oil, CLO: cod liver oil, BO: boost oil, FO fish oil. HIS: hepatosomatic index (x10), LF: % liver fat.

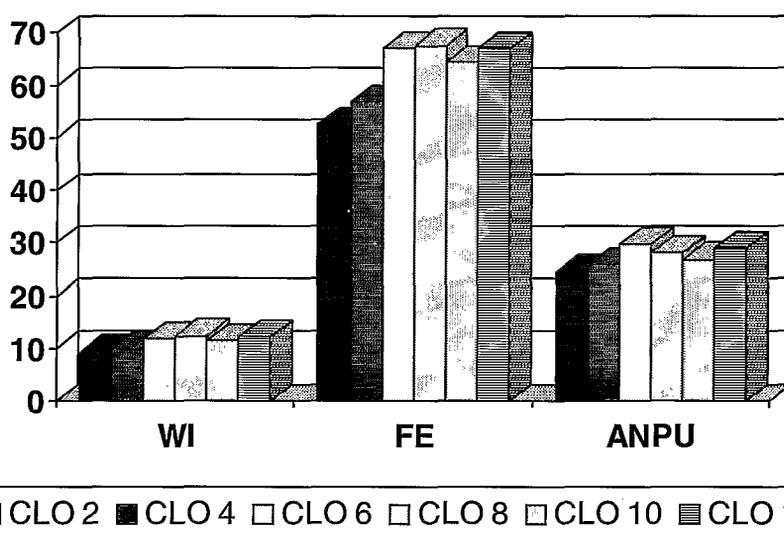


Fig. 9. Performance factors of fish fed diets containing different levels of soybean oil (SBO) and cod liver oil (CLO). WI: % weight gain of fish/10, FE: % feed efficiency, ANPU: apparent net protein utilisation.

(Gatesoupe et al. 1977, Leger et al. 1979), and red sea bream requiring 0.5 DHA or 1% EPA (Takeuchi et al., 1990). The requirements of gilthead bream appear to be at the higher range since the OO/BO diet which contained about 0.65% EPA and DHA indicated a lower performance and inferior liver composition to that of fish fed the fish oil diet.

At low levels of n-3 PUFA supplementation both the growth and the physiological condition of sea bream were affected; the most characteristic change being the

accumulation of lipid in the liver and an increase in the hepatosomatic index of fish (Figure 8). Similar effects have also been observed for other fish species (Watanabe, 1982). The liver is the main organ affected by fatty acid deficiency and this was clearly obvious from the histological examination of liver samples. Diets containing soybean and olive oil as the only lipid sources displaying a clear degeneration of liver cells, with cells having different sizes due to lipid accumulation, broken membranes and concentrations of macrophage cells accumulating in places indicating hepatocyte necrosis. These symptoms were also evident for fish fed diets containing low levels of fish oil. However, the extent of tissue damage within these fish was much lower; degeneration of liver cells was changed to an infiltration of cells by lipid as the fish oil level in the diet increased. Liver infiltration is also a common histological finding within commercially reared fish. Finally fish fed diets containing only plant oils also displayed signs of intense pericarditis and endocarditis. These symptoms were also apparent, although to a much lower degree, within fish fed the low fish oil diets.

Dietary fatty acid composition also influenced the fatty acid composition of the neutral lipids of the fish (Tables 11 and 12). This finding is in accordance with studies for other fish species (Castledine and Buckley, 1980; Dosanjh et al., 1984). The response to dietary treatment resulted in a considerable increase of tissue 18:2n6 as dietary n-6 levels increased, reaching 25-30% of the neutral lipids at the highest dietary n-6 supplementation of 44% during the second experiment. Similarly, during the first experiment where a diet supplemented only with SBO was used (with a 18:2n-6 content of 50% of the total fatty acids) the levels attained within tissue neutral lipids were even higher, ranging from 30 to 44%. A similar response has also been found by Yu and Sinnhuber (1976) for rainbow trout fed different levels of n-3 and n-6 fatty acids; these authors reporting an increase of total n-6 fatty acids up to 40% of body neutral lipids when dietary supplementation reached 50% of the dietary lipids.

Table 11. Fatty acid composition of muscle neutral lipids of gilthead bream fed diets containing different plant oils and fish oils.

	OO	SBO	OO/CLO	OO/BO	FO
Saturates	17.8	16.2	20.1	20.3	27.2
Sum n-9.	69.8	28.7	60.7	57.5	42.3
Sum n-6	8.2	44.6	6.6	5.2	3.4
Sum n-3	1.2	3.9	4.3	5.6	14.0
EPA+DHA	0.2	0.5	2.2	2.5	8.6

The levels of EPA and DHA within the muscle of fish also changed in direct relation to the dietary levels. Since these fatty acids are considered important for human nutrition, their concentrations within the final aquaculture product should also be maintained at satisfactory levels through dietary manipulation.

Table 12. Fatty acid composition of muscle neutral lipids of gilthead bream fed diets containing reciprocating amounts of soybean oil and cod liver oil

CLO %	2	4	6	8	10	12
Saturates	19.4	19.9	20.9	21.8	24.6	25.0
Sum n-9	29.4	29.6	30.3	28.7	29.5	28.4
Sum n-6	35.6	31.4	26.2	21.3	12.9	6.0
Sum n-3	8.3	8.2	10.4	12.8	12.7	16.1
EPA+DHA	2.6	3.3	5.2	7.0	7.5	10.6

Sea bass

By contrast, sea bass performance was not affected by the inclusion of plant oils in the diet, with fish doubling their weight and attaining a final weight of about 200g, and feed efficiency averaging 44% and ANPU 17%. However histological examination of liver tissue revealed liver degeneration, the most intense being in fish fed the diets only supplemented with plant oils (degeneration being less severe for fish fed mixtures of both plant oils and fish oils). In comparison to the plant oil containing diets, the fish oil containing diet only showed signs of fatty infiltration. These results suggest that the EPA+DHA requirements of sea bass might be higher than that of sea bream with the requirement level being about 10% of dietary fatty acids or 1.3% of the diet. The observed fatty acid composition of fish muscle tissue was also directly affected by dietary lipid composition (Table 13) in a manner similar to that observed for sea bream.

Table 13. Fatty acid composition of muscle neutral lipids of sea bass fed diets containing different levels of plant oils and fish oil

	O.O	SBO	SBO/ FO	OO/FO	FO
Saturates	22.0	21.7	24.5	25.1	24.9
Monoenes	57.4	36.9	44,3	51.7	46.8
Sum n-6	10.8	30.0	13,7	10.0	8.7
Sum n-3	9.4	11.1	17.0	12.6	19.2
EPA+DHA	6.5	5.9	12.8	9.4	14.9

Comparison of fatty acid composition of wild and cultured fish

A comparison of the fatty acid composition of wild and cultured fish indicated significant differences regarding the type and level of n-6 fatty acids contained in their tissues, with wild fish containing higher concentrations of n-6 fatty acids which were mainly contributed by arachidonic acid (20:4n-6; Table 14). Linoleic acid

(18:2n-6) was the main n-6 fatty acid present in the lipids of cultured fish originating from the lipids of their diets.

Fatty acids with 20 carbon atoms in their chains such as 20:4n-6 and 20:5n-3 also act as substrates for the production of eicosanoids (prostanoids and leukotrienes), which are potent biological compounds acting as local hormones engaged in the regulation a wide range of physiological processes (Sargent et al., 1989). The pattern of eicosanoids produced by mammalian cells is known to be profoundly affected by the relative abundance of n-6 and n-3 PUFA present in the cellular phospholipids (Weber, 1990). Studies with Atlantic salmon have established that the ratios of n-3 to n-6 PUFA in the phospholipids of leukocytes and gill cells are sensitive to dietary modification as are the profiles of eicosanoids produced by the cells (Bell et al., 1993a,b). For this reason the marked difference observed here between wild and cultured fish in their 20:4n-6/20:5n-3 ratios suggests that differences may also exist between the two groups of fish in the pattern of eicosanoids produced to control basic physiological functions. It follows therefore that the EFA requirements of sea bass and sea bream may require re-examination in the light of the considerable differences observed between wild and cultured specimens.

Table 14. Fatty acid composition of polar liver lipids of wild and cultured sea bream and sea bass (E. and M. refer to the place of collection of fish, Evoikos gulf and Messolonghi lagoon respectively)

	Sea bream		Sea bass		
	Wild		Cultured	Wild	
	E.	M.		M.	Cultured
18:2n-6.	0.7	0.7	4,5	0.5	3.5
20:4n-6.	8.8	12.5	2,6	9.8	1.8
Sum n-6	11.5	18.2	7.9	14.4	7.5
20:5n-3	6.4	8.9	7.7	9.2	11.1
Sum n-3	49.1	40.9	44.0	44.6	41.9

CONCLUDING REMARKS

Fish meal replacers

- Properly heated full fat soya appears to be a good protein source for sea bream. The nutritional value of soybean meal is inferior and is possibly due to the lower heat treatment of the product. Soya protein concentrate was found to have a low nutritional value for sea bream.
- The source and type of treatment of poultry by-products considerably effects their nutritional value and therefore their maximum level of inclusion in sea bream diets

- High quality poultry meal can replace significant amounts of fish meal protein. Feather meals can be used as a feed ingredient but at much lower concentrations
- Meat and bone meals can substitute almost half of fish meal protein without adversely effecting fish performance.

Fish oil replacers

- Substitution of fish oils with plant oils can be performed up to certain levels after which considerable problems in fish performance and/or health develop.
- Fish fatty acid composition is directly related to dietary composition.
- The differences in n-6 fatty acid composition between wild and cultured fish indicate that there might be a need for re-evaluating the fatty acid requirements of Mediterranean fish species.

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