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Effect of dietary lipid sources on the growth and fatty acid composition of gilthead seabream (*Sparus aurata*)

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SUMMARY - Four experimental diets were formulated; a fishmeal-based control diet containing 9% supplemental fish oil, and three diets in which 50% of the supplemental fish oil was substituted with either soybean oil, linseed oil or rapeseed oil (diet 1, 2, and 3, respectively). The diets were fed to fingerling gilthead bream (*Sparus aurata*) over a 45-day experimental test period. Growth and survival were the highest in fish fed the control diet, followed by fish fed diet 1, 2 and 3, respectively. As expected the fatty acid composition of the fish carcass of individual dietary treatments reflected the fatty acid profile of the dietary lipids fed. On the basis of these results it can be concluded that the best plant lipid source tested was soybean oil, followed by linseed oil and rapeseed oil. Considerable savings in feed costs could be achieved if soybean oil, and to a lesser extent linseed oil, could be used as a partial dietary substitute for fish oil within compound feeds for gilthead bream.

Key words: Lipids, Fatty acids, Fish oil, Soybean oil, Linseed oil, Rapeseed oil.

RESUME - "Effet des sources lipidiques du régime sur la croissance et la composition en acides gras de la daurade (*Sparus aurata*)". Quatre régimes expérimentaux ont été formulés : un régime témoin à base de farine de poisson contenant 9% d'huile de poisson supplémentaire, et trois régimes dans lesquels 50% de l'huile de poisson supplémentaire avait été remplacée par soit de l'huile de soja, de l'huile de lin ou de l'huile de colza (régimes 1, 2 et 3 respectivement). Ces régimes ont été distribués à des alevins de daurade (*Sparus aurata*) pendant la durée d'un essai expérimental de 45 jours. La croissance et la survie ont été les plus élevées chez les poissons recevant le régime témoin, suivis par les poissons des régimes 1, 2 et 3 respectivement. Comme on s'y attendait, la composition en acides gras des carcasses de poissons par rapport aux régimes individuels, a reflété le profil en acides gras des lipides du régime qu'ils recevaient. Sur la base de ces résultats, on peut conclure que la meilleure source de lipides d'origine végétale testée a été l'huile de soja, suivie par l'huile de lin et l'huile de colza. Ainsi il pourrait y avoir une réduction considérable des coûts d'alimentation si l'on pouvait utiliser dans le régime de l'huile de soja, et dans une moindre mesure, de l'huile de lin comme produits pour le remplacement partiel de l'huile de poisson dans les aliments composés pour daurade.

Mots-clés : Lipides, acides gras, huile de poisson, huile de soja, huile de lin, huile de colza.

INTRODUCTION

Gilthead seabream (*Sparus aurata*) is an important aquaculture species in the Mediterranean region because of its fast growth rate and delicious taste. However,

the availability of suitable high quality feed is one of the major constraints limiting the growth of the industry. Within many countries 'trash fish' is still being used as a food source for culture operations. However, since the supply of 'trash fish' is usually both limited, expensive and seasonal in most countries within the region, there is an urgent need to develop alternative feeding methods and in particular to develop cost effective artificial feeds and increase productivity. Despite this, need there is scant information on the dietary nutrient requirements of gilthead bream over the various stages of its life cycle, and in particular concerning lipid nutrition. For example, dietary lipids are generally highly digestible and appear to be favoured over dietary carbohydrates as an energy source in fish (Halver, 1976; Cowey and Sargent, 1977; Cho, Cowey and Watanabe, 1985).

In contrast to freshwater omnivorous fish species, marine carnivorous fishes have a very limited ability to chain elongate and desaturate fatty acids and thus require a dietary supply of 20:5 n-3 and/or 22:6n-3 fatty acids in a ready made form to satisfy their dietary fatty acid requirements. Some plant oils, such as linseed oil and soybean oil contain significant quantities of n-3 fatty acids (Lovell, 1994). Moreover, although linseed oil is currently only produced in small quantities, it is a rich source of both 18:3 n-3 and 18:2 n-6 (Tocher, et al., 1990).

Although fish oil is an excellent source of n-3 fatty acids for fish, for many countries it is both unavailable and costly to import. The aim of the present study was therefore to investigate the possibility of replacing 50% of the fish oil normally used within diets for gilthead seabream with different sources of plant lipids (namely, soybean oil, linseed oil, and rapeseed oil) and to monitor the changes in the growth and fatty acid composition of fingerlings fed these diets.

MATERIAL AND METHODS

Rearing facilities and water quality

The feeding trial was carried out at the Demitee Governate Fish Farm in Egypt using Gilthead seabream fingerlings of average initial weight 22.5g (total length 7.01cm). Fish were kept under continuous observation for one week prior to the start of the experiment and then randomly distributed within 12 glass aquaria (each aquarium having a 90-litre water capacity) at a stocking density of 12 fish per aquarium, with three replicates per dietary treatment. Water salinity (32 ppt), temperature (24°C) and saturated oxygen levels were maintained within the experimental tanks for the duration of the 45-day feeding trial.

Experimental diets and feeding regime

Four iso-calorific and iso-nitrogenous diets were formulated; a control fish-meal based diet supplemented with 9% fish oil (control), and three diets in which 50% of the fish oil was substituted with soybean oil (diet 1), linseed oil (diet 2), and rapeseed oil (diet 3). The formulation and proximate composition of the experimental diets is shown in Table 1 and the fatty acid composition of the diets shown in Table 2. All the experimental diets were prepared in moist pelleted form

using a kitchen mincer. Fish were fed the experimental test diets at a fixed rate of 10 % body weight per day (the feed divided into four equal parts over the working day), and the feeding rate adjusted at weekly intervals for the duration of the 45-day experiment.

Chemical analyses

Proximate analyses for moisture, crude protein, crude fat, crude fibre and ash were determined using the standard methods of AOAC (1970).

Lipids and fatty acids

Experimental diets and fish samples from each aquarium were analysed for total lipids and fatty acids at the start and end of the experiment. Lipids were extracted from the samples according to the method of Bligh and Dyer (1959) and the extracted lipids then subjected to GLC analysis; the lipids being saponified according to the method of Ashour (1991) and the fatty acids methylated with ethereal diazomethane as recommended by Vogel (1954). A Perkin - Elmer GLC (series 8300) was used for detecting the fatty acids.

Table 1. Composition of the experimental diets (% by weight)

Ingredients	Control	Diet 1	Diet 2	Diet 3
White fish meal (imported)	63.54	63.54	63.54	63.54
Corn meal	15.46	15.46	15.46	15.46
Fish oil	9.00	4.50	4.50	4.50
Soybean oil	-	4.50	-	-
Linseed oil	-	-	4.50	-
Rapeseed oil	-	-	-	4.50
Vitamin mix ¹	3.00	3.00	3.00	3.00
Mineral mix ²	3.00	3.00	3.00	3.00
Molasses (binder)	6.00	6.00	6.00	6.00
Total	100	100	100	100

Proximate composition (%)

Moisture	9.60	9.00	9.00	9.00
Crude protein	58.6	58.7	58.5	58.5
Crude fat	10.2	10.0	10.0	10.0
Crude fibre	3.2	3.0	2.9	3.3
Nitrogen free extract	14.4	14.6	15.0	14.6
Ash	4.0	4.7	4.6	4.6

¹ Vitamin mix. (g/kg): Vit. A (0.012), Ergocalciferol (0.006), Tocopheral (0.40), Menadione (0.04), PABA (0.4), Biotin (0.006), L-ascorbic acid (3.0), D-calcium pantothenate (0.28), choline chloride (8.0), Folic acid (0.015), Myo-inositol (4.0), Niacin (0.80), Pyridoxine HCl (0.04), Riboflavin (0.20),Thiamine HCl (0.06), BHT (0.30), Celuflil (12.44)

² Mineral mix (g/kg): Ca (H₂PO₄)₂, H₂O (0.40), Ca lactate (1.0), Fe Citrate (0.10), MgSO₄.7H₂O (0.40), K₂HPO₄ (0.70), NaH₂PO₄, H₂O (0.25), AlCl₃.6H₂O (0.02), ZnSO₄.7H₂O (0.126), CuSO₄.5H₂O (0.03), MnSO₄.H₂O (0.015), KI (0.02), CaCl₂. 6H₂O (0.02), celuflil (12.44).

Table 2: Fatty acid composition of the experimental diets (% by weight of total fatty acids)

Fatty acids*	Control	Diet 1	Diet 2	Diet 3
14 : 0	5.8	1.22	5.62	3.43
15 : 0	0.5	0.14	3.38	1.73
16 : 0	7.7	9.06	5.45	6.30
16 : 1	7.2	9.96	18.62	12.29
16 : 2	1.4	0.18	0.72	3.45
18 : 0	1.9	5.14	9.19	8.16
18 : 1 n-9	22.3	23.89	18.90	16.39
18 : 2 n-6	12.2	32.82	19.75	26.20
18 : 3 n-3	7.0	5.53	6.82	5.82
18 : 4 n-6	1.5	0.29	1.05	1.17
20 : 1 n-9	14.8	5.39	3.38	4.38
20 : 4 n-6	1.5	0.71	1.03	0.73
20 : 5 n-3	4.9	2.26	2.60	2.16
22 : 4 n-6	1.2	0.16	0.12	2.14
22 : 4 n-3	2.0	0.53	0.43	1.07
22 : 5 n-6	1.6	0.32	0.04	2.18
22 : 5 n-3	1.7	0.15	0.09	0.12
22 : 6 n-3	4.7	2.13	2.06	2.10
Total Saturated.	15.9	15.56	23.64	17.64
Total Unsat. FAS	84.1	84.31	75.61	79.84
Total PUFAS	38.3	44.90	33.99	43.33
Total n-3 FAS	20.3	10.60	12.00	11.27
Total n-6 FAS	18.0	34.30	21.99	32.06
n-3 / n-6 FAS	1.13	0.31	0.55	0.35

*: Fatty acids are designated by chain length: number of double bonds : double bond closest to the terminal methyl end.

Statistical analyses

Statistical analyses were performed by IBMAT; computer through the statistical analyses system SAS (Barr *et al.* 1976).

RESULTS AND DISCUSSION

The fatty acid composition of the fish carcass at the beginning and end of the experimental feeding trial is shown in Tables 3. As expected the carcass fatty acid profiles of the fish at the end of the experiment reflected the fatty acid profiles of the experimental diets fed. Similar results have also been obtained for other freshwater and marine fish species (Yu, Sinnhuber and Putnam, 1977, Yingst and Stickney, 1979; Reinitz and Yu, 1981). It was also apparent that a proper balance of dietary

fatty acids is extremely important to ensure rapid growth in Gilthead bream fingerlings. Although the soybean oil-based diet (diet 1) had low levels of arachidonic acid (20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3, Table 2), fish fed this ration had surprisingly the highest level of EPA at the end of the experiment; suggesting possible chain elongation and desaturation of linolenic acid (18:3n-3) in these fish.

Table 3: Fatty acid composition of carcass lipids of gilthead bream at the beginning and end of the 45-day feeding trail (values expressed as % by weight of total fatty acids)*

Fatty acids	Initial	Control	Diet 1	Diet 2	Diet 3
14 : 0	1.14	1.69	2.54	3.93	2.31
15 : 0	0.67	0.51	0.20	0.39	0.98
16 : 0	12.07	14.83	15.28	22.96	22.67
16 : 1	6.76	9.81	6.54	12.95	11.35
16 : 2	2.44	1.56	0.54	0.92	0.63
18 : 0	22.18	19.07	14.99	15.32	18.54
18 : 1 n-9	17.57	18.39	18.83	19.64	17.85
18 : 2 n-6	6.35	1.66	6.91	1.34	2.89
18 : 3 n-3	3.37	6.49	6.13	4.31	3.42
18 : 4 n-6	0.95	0.62	1.64	0.54	1.14
20 : 1 n-9	7.93	3.27	4.87	5.33	2.18
20 : 4 n-6	1.75	0.42	1.72	0.55	1.81
20 : 5 n-3	5.15	6.37	4.78	1.75	2.63
22 : 4 n-6	0.51	0.98	2.76	1.82	4.33
22 : 4 n-3	0.81	2.15	1.78	2.75	2.36
22 : 5 n-6	1.90	1.59	2.23	1.85	1.55
22 : 5 n-3	4.45	3.19	3.94	2.43	2.15
22 : 6 n-3	2.13	7.38	4.32	1.24	2.21
Total sat. FAS	36.06	36.12	33.01	42.60	44.50
Total Unsat. FAS	62.07	63.88	66.99	57.39	56.5
Total PUFAS	36.17	30.55	36.21	18.58	24.49
Total n-3 FAS	15.91	25.28	20.95	12.27	12.77
Total n-6 FAS	11.46	5.27	15.26	6.31	11.72
n-3 / n-6 FAS	1.39	4.79	1.39	1.94	1.08

*Average of triplicate groups.

The growth response of fish fed the experimental diets is shown in Table 4. As expected, the highest weight gain and growth was observed for fish fed the control fish oil-based diet, closely followed by fish fed the rations supplemented with soybean oil (diet 1), linseed oil (diet 2), and rapeseed oil (diet 3). However, it was also apparent from these results that the growth differences between treatments was very small (especially between fish fed the control ration and diet 1); indicating that

plant lipids can substitute part of the dietary fish oil component of feeds for fingerling Gilthead seabream with no significant loss in fish performance.

Table 4: Weight gain and growth of Gilthead seabream fed the experimental diets for 45 days

Parameter	Control	Diet 1	Diet 2	Diet 3
Initial body weight (g)	22.50 ± 2.9 a			
Final body weight (g)	86.68 ± 6.02 a	85.62 ± 4.91 a	84.52 ± 5.11 b	82.68 ± 5.05 c
Mean final weight gain (g)	64.18 ± 0.12a	63.12 ± 2.01b	62.02 ± 2.21b	60.18 ± 2.15c
Weight gain %	185.24	180.53	175.64	167.47
SGR % / day	2.14 a	2.12 a	2.08 b	2.02 c

$$\text{SGR \% / DAY} = \frac{\text{Ln final weight} - \text{Ln initial weight}}{\text{No. of days}} \times 100 \quad (\text{Degane and Viola, 1987})$$

CONCLUSIONS

On the basis of the encouraging results obtained during this feeding trial, it is clear that soybean oil, and to lesser content linseed oil, offer considerable potential for use in aquafeeds as a source of dietary energy and essential fatty acids for Gilthead seabream. However, although these plant lipids may be more available and/or cheaper than fish oil in some countries, considerable further research is required concerning their long term use in aquafeeds and concerning their possible effects on flesh quality.

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