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Partial replacement of fish meal by T-Iso in gilthead sea bream (*Sparus aurata*) juveniles diets

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ABSTRACT - The aim of this study was the evaluation of microalga *Isochrysis sp.* T-Iso in partial substitution of fish meal and the study of the effects on gilthead sea bream (*Sparus aurata*) performances and chemical composition of fillets. The results show that the microalga T-Iso nutrients support growth better than control diets, and the chemical composition of sea bream fillets also meets the needs of consumers for healthy diets. T-Iso resulted highly digestible, and support the best performances of fish fed on 70% alga diet probably due to its high protein efficiency in comparison to other diets. The presence of a high quantity of cyclic isoprenoid could explain this high efficiency of T-Iso. Gilthead sea bream fed on 70% T-Iso showed fillets with a low level of protein and a high level of fat; moreover, their somatic indexes were higher than those of fish fed other diets. Highest percentage T-Iso diet showed the highest amount of the sum of saturated fatty acids, mainly due to myristate and palmitate. On the contrary, the sum of polyunsaturated decreases, mainly because of the reduction of EPA and DHA amounts. If the mass production economical problems are solved, T-Iso will represent a good solution in partial substitution of fish meal.

Key words: T-Iso, *Sparus aurata*, Fatty acid, Fillet composition.

Introduction - The current trend in fish farming is towards increased use of plant protein in commercial fish diets. In the last decade, much more attention has been paid to plant source of protein (Palmegiano *et al.,* 2006). Microalgae are increasingly being used as feed in aquaculture for many marine organisms, because they constitute a source not only for energy, but also of many essential compounds such as the polyunsaturated fatty acids (Belay *et al*., 1996). The possible use of microalgae has been investigated as a nutrient for fish larvae as well as for juveniles and adults (Lu and Takeuchi 2004; Palmegiano *et al.,* 2008). Among those algae, *Isochrysis sp.* showed to have high quality of nutrients (CP: 43.0; EE: 18.7; Ash: 10.1; Crude fiber: 0.9; Total starch: 10.4 (99% gelatinized) and to be a good source of polyunsaturated fatty acids and in particular docosahaesenoic acid (DHA) (Trotta, 1998). The aim of this study was to evaluate a microalga (*Isochrysis sp.* T-Iso) as source of plant protein and ether extract in partial substitution of fish meal and fish oil, studing its effects on gilthead sea bream (*Sparus aurata* L.) performances and chemical composition of fillets.

Material and methods - Two hundreds and fourthy gilthead sea bream (22.3±0.3 g) were randomly distributed in 12 tanks (100 l; water temperature constant at 18°C). Four diets were compared:

two experimental diets containing respectively 30% and 70% (on wet weight basis) of freeze-dried T-Iso and two control diets: the first as from a commercial diet for trout (closed formula) and the other one an extruded diet (closed formula). Diets were allocated to triplicate fish groups according to a completely random experimental design. Fish were fed 1.2% of their body weight. Proximate analysis of algal masses and diets have been performed according to the AOAC methods (2000). Lipids, for the determination of fatty acid composition, were extracted according to Folch *et al.* (1957). Fatty acid methyl esters (FAME) were prepared by methylation procedure (AOAC 2000) and were separated and quantified by gas chromatography (Perkin Elmer 8700). Fish were individually weighted at the beginning and at the end of the trial and in order to adjust the feeding quantities weighed in bulk every 15 days. The trial lasted 8 weeks and at the end performance indexes were calculated. Moreover, proximate fillet composition were determined following AOAC (2000) and viscera and hepatosomatic index were determined. Gross energy (GE) of diets and fillets samples was determined utilizing a calorimeter Ika C7000 (Heitersheim, Germany). Results were expressed as percentages and ratios were transformed in arcsin of roots; on transformed and normalized data variance analyses were performed by one way analysis of variance (ANOVA); significant differences were ranged (P≤0.05) by Duncan test.

Results and conclusions - The chemical composition of the diets is reported in Table 1.

Fish readily accepted all experimental diets. Productive trait indexes (Table 2) showed a better individual and biomass growth in sea bream fed on diet with higher microalga content. The T-Iso cellular wall is thin and make its nutrients more useful for sea bream to energy deposition. The relatively high gelatinised amount of carbohydrates well balances the protein energy ratio.

Growth performances resulted in agreement with other studies on the same species (Lupatsch *et al.*, 2001). The best performances of fish fed on 70% alga diet are probably due to its high protein efficiency in comparison to other diets (2.0 vs 1.3-1.5), despite its lowest protein energy ratio. The little size (Ø≤70 μ) of the cells of T-Iso in comparison to the higher mesh of the other feedstuffs (Ø≈250 μ) could make easy the attack of the digestive juice, and consequently the absorption of the nutrients. VSI values are higher in extruded diet and in 70% alga diet than the values of the other two diets (4.9 and 5.0 vs 3.8 and 3.4

Table 1. Chemical composition of diets (mean ±sd).

	Extruded diet			Basal diet			30% T-Iso			70% T-Iso		
CP	49.5	±	0.01	49.1	±	0.4	43.9	±	0.03	41.4	±	0.1
EE	19.5	±	1.4	6.1	±	0.9	8.4	±	0.3	11.8	±	1.1
Ash	11.4	±	0.03	11.8	±	0.3	10.9	±	0.01	9.6	±	0.2
GE (Mj/kg)	21.6	±	0.01	19.1	±	0.03	19.2	±	0.03	20.2	±	0.01
DP/DE	2.9	±	0.01	2.6	±	0.02	2.3	±	0.01	2.0	±	0.01

Table 2. Productive traits during the experiment (8 weeks) (mean ±sd).

	Extruded diet			Basal diet			30% T-Iso			70% T-Iso		
Initial weight (g)	22.3	±	0.2	22.9	±	0.4	22.6	±	0.1	22.5	±	0.1
WG	6.9	±	0.9[b]	7.1	±	0.4[b]	7.3	±	0.6[b]	9.1	±	1.4[a]
FCR	1.6	±	0.1[a]	1.7	±	0.2[a]	1.5	±	0.1[a]	1.2	±	0.1[b]
SGR	0.9	±	0.1[b]	0.9	±	0.1[b]	0.9	±	0.1[ab]	1.1	±	0.2[a]
PER	1.3	±	0.1[b]	1.4	±	0.1[b]	1.5	±	0.1[b]	2.0	±	0.2[a]
HSI	1.1	±	0.4	0.8	±	0.6	1.0	±	0.2	1.3	±	0.3
VSI	4.9	±	0.8[a]	3.8	±	1.8[b]	3.4	±	0.1[b]	5.0	±	1.2a

[a,b]: P <0.05; WG=mean individual final weight–mean individual initial weight; FCR=total feed given (g of DM)/WG (g); SGR (% per day)=[(ln final weight-ln initial weight)x100/t]; PER=WG (g)/amount of protein fed (g); HSI=(liver (g)/body in weight (g))x100; VSI=(viscera (g)/body in weight (g))x100.

respectively). No statistically differences appeared for chemical composition of fillets (mean ± sd values: CP 49.6±1.7, EE 20.9±1.3, Ash 13.8±0.8, GE 22.4±0.7). As lipid composition of fillet are concerned (Table 3), differences in fatty acid profile were observed. In particular, the sum of saturated fatty acid increases in diets integrated with microalgae; this effect is mainly due to the high content in myristate (C14:0) and palmitate (C16:0). On the contrary, the sum of polyunsaturated decreases, mainly because of the reduction of Eicosapentaenoic acid (EPA - C20:5n-3) and Docosahaesenoic acid (DHA – C22:6n-3) amounts.

The decreasing of fillet linolenic acid (from 3.4% in 70% alga diet to 0.3% in fillet and from 1.6% in 30% alga diet to 0.3% in fillet) could mean that in fillets of gilthead sea bream fed T-Iso diets this fatty acid is elongated and desaturated, and transformed in EPA and in a lesser way in DHA.

The results of this study show that the microalga T-Iso nutrients support growth better than control diets, and the chemical composition of sea bream fillets also meets the needs of consumers for healthy diets. T-Iso resulted highly digestible, and support the best performances of fish fed on 70% alga diet are probably due to its high protein efficiency in comparison to other diets. Sea bream fed on 70% T-Iso showed a low level of protein and a high level of fat; moreover, their somatic indexes were higher than those of fish fed other diets.

Highest percentage T-Iso diet showed the highest amount of the sum of saturated fatty acids, mainly due to myristate and palmitate. On the contrary, the sum of polyunsaturated decreases, mainly because of the reduction of EPA and DHA amounts. If the mass production economical problems are solved, T-Iso will represent a good solution in partial substitution of fish meal.

Table 3. Fatty acid profile of fillets (% of total fatty acids, mean ±sd).

	Extruded diet	Basal diet	30% T-Iso	70% T-Iso
C14:0	8.1 ± 0.6	7.7 ± 0.3	9.0 ± 0.3	9.7 ± 0.2
C16:0	26.9 ± 0.6^{ab}	26.2 ± 0.9^{b}	27.5 ± 1.7^{ab}	27.9 ± 1.5^{a}
C16:1	6.0 ± 0.3	6.1 ± 1.0	6.0 ± 0.4	5.8 ± 0.6
C18:0	5.1 ± 0.5	5.1 ± 0.5	5.2 ± 0.5	5.2 ± 0.7
C18:1	17.3 ± 1.0^{b}	18.7 ± 0.6^{a}	18.4 ± 0.0^{ab}	17.6 ± 1.8^{ab}
C18:2n6	1.2 ± 0.1	1.5 ± 0.3	1.1 ± 0.1	1.1 ± 0.3
C18:3n6	0.3 ± 0.0	0.8 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
C20:5n3	5.1 ± 0.3	5.1 ± 0.4	4.7 ± 0.2	4.5 ± 0.5
C22:6n3	6.3 ± 0.4	6.2 ± 0.2	5.2 ± 0.0	4.7 ± 0.4
SFA	44.7 ± 0.1	44.5 ± 1.7	46.4 ± 0.4	48.5 ± 2.3
MUFA	27.9 ± 0.3	29.8 ± 1.6	28.6 ± 0.7	27.6 ± 2.6
PUFA	19.1 ± 0.6^{a}	18.7 ± 0.0^{ab}	17.3 ± 0.0^{b}	17.5 ± 0.3^{b}
DHA/EPA	1.2 ± 0.0^{a}	1.2 ± 0.0^{a}	1.1 ± 0.0^{ab}	1.1 ± 0.0^{b}

^{a,b}: *P <0.05*

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