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Impacts of lipids and (n-3) exceptionally unsaturated fats organizations of three counterfeit nourishments on endurance, development and body sythesis of normal dentex's fingerlings

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Abstract

Dentex juveniles of initial live weight 5 - 6 g and 108 days old after hatching, were fed three different diets for ten weeks (moist pellet (MP), imported dry pellet (IDP) and locally dry pellet (LDP)) containing three different lipid levels (26, 21 and 10% respectively) and crude protein levels (46, 45 and 47% respectively). Fatty acid composition showed that, DHA and EPA contents of moist pellet are higher than those of imported and locally dry pellets. DHA/EPA ratios of muscle were of 1.04 ± 0.00 (MP), 1.48 ± 0.57 (IDP) and 0.72 ± 0.05 (LDP). They increased in fact during feeding period for both the three tested diets while muscle's EPA content decreased compared with its initial state when beginning this experiment. Obtained specific growth rate (SGR) by weight were 1.7, 2.4 and 2.7% and food conversion rate (FCR) were 1.8, 1.3 and 1.5 respectively for MP, IDP and LDP. While survival did not show significant difference (P>0.05), the best growth performance was observed using IDP and LDP as fish fed diets containing 47/10 and 45/21 protein/lipid ratios.

Keywords: *Dentex dentex*, fingerlings, lipids, (n-3) HUFA, survival, growth**.**

INTRODUCTION

The common Dentex (*Dentex dentex*) is a marine sparid fish specie with high commercial value. It is considered as a potential candidate for aquaculture, aiming to diversify Mediterranean Aquaculture production (Conides et al., 1996; Conides and Nengas, 1998). Captive breeding have been undertook since two decades; high growth was observed during larval phase (Abellan et al., 1997; Tulli et al., 1997; Koumoundourous et al., 2004), fingerlings stage and larger fish (Efthimoiou et al., 1994; Company et al., 1999; Katavic et al., 2000; Koumoundourous et al., 2004). The Dentex's fingerling feeding requirements have been studied (Tibaldi et al., 1996; Cardenete et al., 1997a, b, c; Company et al., 1999) using pellet's diets for juveniles weighing 10 - 30 g, but dietary lipid and protein steel need improvement. Riera et al. (1993) have achieved experimental Dentex's fingerlings grow-out in sea cage feeding on natural food and obtained adult fish weight of 830 g in 16 - 17 months, widely superior to growth of cultured Sea bass and gilt- head bream. Lipids are known to play an important role in fish nutrition by supplying energy and essential fatty acids (Sargent et al., 1989; Parpoura and Alexis, 2001). A lot of importance was granted in the effect of fatty acids composition on the quality of eggs, larvae juveniles. Aiming to contribute to master Dentex pre- ongrowing feeding phase, this study was focused on the influence of lipids and fatty acids on survival, growth and body composition of its fingerlings.

MATERIAL AND METHOD

Fingerling's culture

Locally hatchery produced fingerlings of common Dentex have been used in this experiment. They were reared during ten weeks in six 500 L fibreglass cylindro-conical tanks. After being sorted and selected to constitute homogeneous groups, 20 fishes were initially

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Table 1. Composition of the three experimental diets (LDP and IDP compositions are given following manufacturers' technical sheet). DM = dry matter; WM = wet matter.

Diet	MP	LDP[a]	IDP[b]
Ingredients			
Dry matter (%)	66	90	92
Fish meal, sardine (%WM) (5-02-015)	40	---	---
Fresh fish (%WM)	38	---	---
Sardine oil (%WM)	18	---	---
Premix vit[c] (%WM)	4	---	---
Crud protein (%DM)	---	50	45
Crud lipid (%DM)	---	12	21
Crud fiber (%DM)	---	1.2	1.5
Ash (%DM)	---	9.8	11
Carbohydrates(%DM)	---	---	14.5
Vit A	---	20000 IU Kg^{-1}	5000 IU Kg^{-1}
Vit D	---	3000 IU Kg^{-1}	1000 IU Kg^{-1}
Vit E	---	250 mg Kg^{-1}	180 mg Kg^{-1}
Vit C	---	350 mg Kg^{-1}	---
Analysed composition (%DM)			
Crud protein	46	47	45
Crud lipid	26	10	21
Ash	11.60	9.50	10.40

[a]Ingredients: fish products, seed oil products and by-product set, cereal seed products, vitamins and minerals, antioxidants : ethoxyquin.
[b]Ingredients: fish meal, soybean, horse bean, pea, fish oil, rapeseed, maize gluten, rapeseed oil, weat, vitamins and minerals, antioxidants : ethoxyquin.
[c]Premix (dose kg^{-1}): Minerals, 75% ; Phosphor, 3 – 4% ; Calcium 25 – 30%, Vitamin A, 2.000000 IU ; Vitamin D 3, 400000 IU ; Thiamine B_1, 500 mg ; Riboflavin B_2, 1.000 mg; Calcium pantothenate B_3, 7500 mg; Pyridoxine B 6, 500 mg; Vitamin B_{12}, 1.5 mg; Tocopherol Acetate E, 2500 mg; Nicotinic Acid PP, 10000 mg; Cholin (Chloride), 50000 mg.

stocked in each tank in such way to have equal biomass in each the six tanks. Initial average weights of these fingerlings were ranged between 5 ± 0.2 and 6 ± 0.2 g. Seawater flow- through system was used with a renewal rate maintained around 4 - 5 times a day (d^{-1}). Oxygenation was provided by two air diffusers and Dissolved Oxygen (DO) was over saturation during the entire period of the experiment. Temperature (17.5 - 22°C), salinity (36 - 38‰) and photoperiod conditions were natural. Light intensity was kept around 600 luxes during the whole experiment. Feeding frequency was twice a day and Dentex's fingerlings were fed to satisfaction.

Survival, length and weight assessment

Survival rate's estimation was based on daily counting of dead fishes and final remaining number of live fishes at the end of experience. Dentex's fingerlings were measured for their total length on the following days (d_0, d_4, d_{14}, d_{21}, d_{34}, d_{47}, and d_{68}). They were anaesthetized using phenoxy-2-éthanol (0.1 mL per liter of seawater). Total length was first assessed using a gradual table from the snout point until the longest beams point of the caudal fin when this one is aligned with the main axis of the body. Then, their individual body weight was taken using an electronic balance. Fingerlings were thereafter put back in their respective rearing tanks.

Sampling for biochemical analysis

Biochemical analysis was made upon both diets and fingerlings muscle. Diets samples were taken one time from moist pellet, locally and imported dry pellets feeding stocks. While fingerlings muscle were taken twice, at the beginning and at the end of the experiment. Initial muscle sampling took place just before starting this feeding experiment and was made on randomly selected 30 dentex's fingerlings. Final muscle sampling was made at the end of this experiment upon 5 fingerlings for each diet group (MP, IDP, and LDP). All samples were frozen before freeze-drying.

Experimental diets and their composition

Three diets were experimented. An imported dry pellet (IDP) basically conceived and used for sea bass, a dry pellet (LDP) which was locally manufactured and a moist pellet (MP) locally prepared using trash fish and fish meal (Table 1).

Biochemical analysis

Proteins, humidity and ashes were determined using standard method (AOAC, 2000). Total lipids were extracted and measured

Table 2. Fatty acid composition (mg g^{-1} DW) of the tested food. Data represent mean ± SEM

Fatty acid	Moist pellet	Local dry pellet	Imported dry pellet
14:0	8.9±0.20[a]	6.5±0.49[b]	11.3±0.17[c]
15:0	5.4±0.01[a]	5.2±0.04[b]	6.0±0.03[c]
16:0	13.1±0.19[a]	14.4±1.64[b]	39.8±1.69[c]
17:0	6.4±0.01[a]	6.4±0.02[a]	8.0±0.02[b]
18:0	3.0±0.01[a]	5.5±0.03[b]	9.1±0.29[c]
16:1	4.4±0.32[a]	5.2±0.21[b]	5.0±0.02[c]
17:1	3.4±0.32[a]	nd	4.1 ±0.00 [b]
18:1n-9c	17.4±0.07[a]	19.2±0.17[b]	10.4±0.35[c]
18:1n-7	6.0±0.08[a]	5.3±0.01[b]	3.3±0.16[c]
20:1n-9	3.2±0.12[a]	1.5±0.09[b]	2.2±0.01[c]
22:1n-9	0.5±0.03	0.3±0.04	0.4±0.16
22:1n-11	nd	nd	nd
24:1n-9	0.1±0.01[a]	0.9±0.14[b]	0.2±0.03[a]
18:2n-6c	7.8±0.18[a]	29.8±0.62[b]	0.2±0.01[c]
20:2n-6	0.3±0.01	0.2±0.02	0.1±0.00
20:4n-6	0.7±0.01[a]	nd	0.1±0.00[b]
18:3n-3	2.8±0.04[a]	3.6±0.16[b]	0.3±0.03[c]
20:3n-3	0.2±0.04[a]	0.3±0.15[b]	0.1±0.13[c]
20:5n-3	10.7±0.06[a]	3.1±0.25[b]	3.2±0.08[c]
22:5n-3	1.2±0.01	nd	nd
22:6n-3	11.1±0.04[a]	4.6±0.53[b]	2.3±0.02[c]
DHA/EPA	1.04±0.00[a]	1.48±0.05[b]	0.72±0.57[c]
n-3 HUFA	23.0±0.32[a]	7.7±0.45[b]	5.5±0.06[c]

n-3 HUFA = N-3 HUFA >20:3n-3; nd = not detected
Means ± SEM having different letters (a, b and c) indicate that treatments are significantly different (P<0.05) according to Neuwman-Keuls test (n = 2).

gravimetrically according to Folch et al. (1957) using dichloromethane instead of chloroform. Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids using boron trifluoride methanol according to Santha and Ackman (1990) and were analysed in a Varian 3,400 gas chromatograph. The chromatograph was equipped with a DB Wax fused silica capillary column (30 m x 0.25 mm i.d., film thickness: 0.25 µm, J and W Scientific, Folsom, CA). Helium was used as carrier gas (1.4 mL min^{-1}) and the thermal gradient was 100 to 180°C at 8°/min, 180 to 220°C at 4°/min and a constant temperature of 220°C during 25 min. Injector (Split/ splitless with automatic pas-sage) and flame ionisation detector temperatures were 260 and 250°C respectively. Fatty acid methyl esters were identified by comparison with known standard mixtures (Sigma ref. 189-13) and quantified using a computer system. Data were collected and processed using the Star computer package (Varian).

Statistical analysis

Total length and body weight were compared by one-way analysis of variance (ANNOVA) followed by Neuwman-Keuls test when test is significant. A significant level of 5% was used for both tests (Sokal et al., 1981). Statistical study was done by STATITFC (ITFC 4.0, 1988); total lengths and weights of fingerlings were submitted to a logarithmic transformation, while the survival rates are treated after an angular transformation.

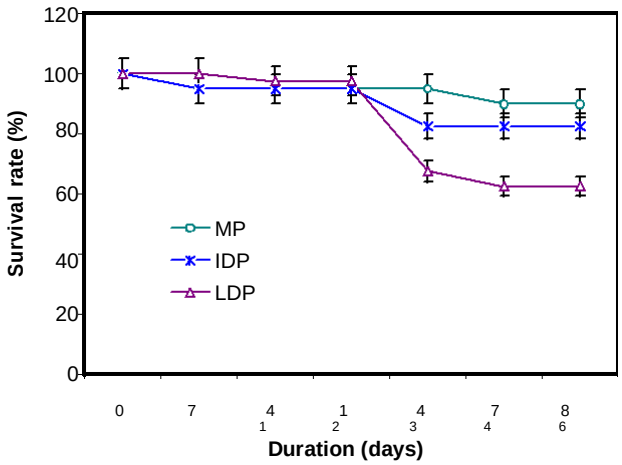

Figure 1. Survival (%) of Dentex's fingerling fed three different diets. Means ± SEM, P>0.05 (ANNOVA test).

RESULTS

Table 2 shows that local pellet composition in 18:3n-3 (3.6 ± 0.16 mg g^{-1} DW) was higher than that of the two other foods while all of them showed a weak content in 20:3n-3. In terms of EPA an DHA contents, MP presented the highest level with respectively 10.7 ± 0.06 mg g^{-1} DW and 11.1 ± 0.04 mg g^{-1} DW followed by LDP (EPA: 3.1 ± 0.25 and DHA: 4.6 ± 0.53 mg g^{-1} DW) and finally IDP (EPA: 0.2 ± 0.08 and DHA: 0.3 ± 0.02 mg g^{-1} DW). The DHA/ EPA ratio was of 1.04 ± 0.00, 1.48 ± 0.05 and 0.72 ± 0.57 respectively for the MP, LDP and IDP diets. Their respective n-3 HUFA contents were of 23.0 ± 0.32, 7.7 ± 0.45 and 5.5 ± 0.06 mg g^{-1} DW.

Figure 1 shows the evolution of *Dentex*'s fingerlings survival fed on the three diets during ten weeks culture. During first three weeks, results of survival showed weak changes, followed thereafter by a relatively high mortality which began on day 22 particularly for IDP and LDP fingerlings groups. The overall survival at the end of this experiment was of 90% for fish's group fed on MP, 82.5% for the one fed on IDP and 62.5% for the one fed on LDP.

Figure 2 showed that Dentex's fingerlings fed on dry pellet (both IDP and LDP) had a better linear growth than those fed on moist pellet (MP). Specific Growth Rate (SGR) of Total Length (TL) was 0.6 for MP, 0.9 for LDP and 0.9 for IDP.

Figure 3 showed that *Dentex*'s fingerlings weight growth follow same trend as their linear growth. Their initial weights were statistically equals in the three experimented diets fish's groups (5.87 ± 0.29 g for fingerlings fed on MP, 6.22 ± 0.34 g for LDP and 5.94 ± 0.38 g for IDP). LDP fish's group gave the highest growth rate (2.7%), follow-up by IDP group (2.4%) and finally by MP group which gave the lowest growth rate (1.7%). The Food Conversion Rate (FCR) was of 1.8, 1.5 and 1.3 respectively for MP, LDP and IDP groups (Table 3).

Table 4 showed that fingerling's muscle initial contents

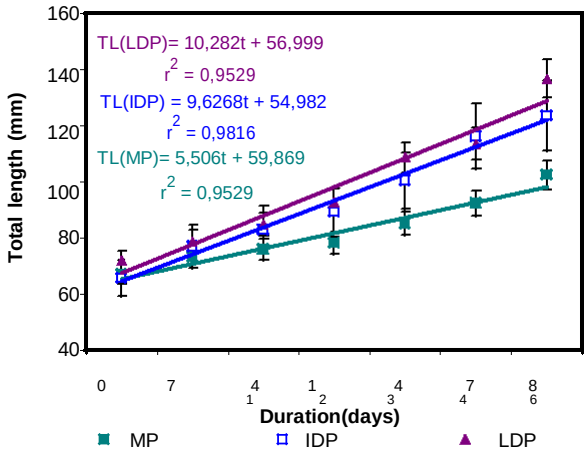

Figure 2. Linear growth curve of Dentex's fingerlings fed three different diets . Mean ± SEM, P<0.05. (Neuwman-Keuls test) (n = 20).

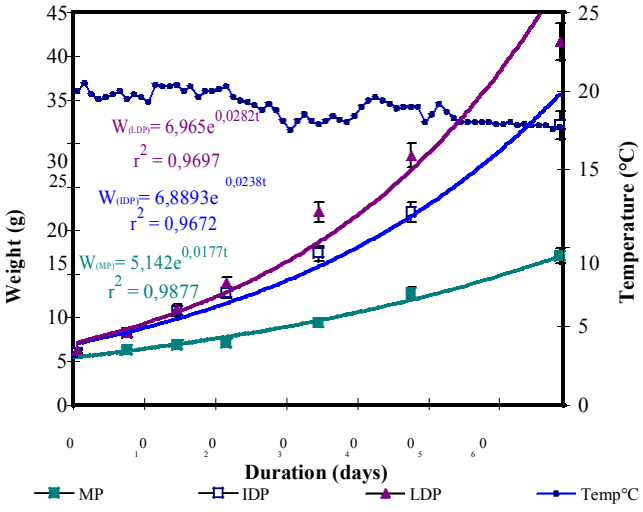

Figure 3. Weight growth curve of Dentex's fingerlings fed three different diets. Means ± SEM, P<0.05. (Neuwman-Keuls test) (n = 20).

in 18:3n-3 and 20:5n-3 were respectively 2.1 ± 0.08 and 11.3 ± 0.23 mg g^{-1} DW. These values are higher than those obtained at the end of the experiment for the three diets fish's groups. DHA muscle's content of fingerlings fed on MP was of 10.8 ± 0.61 mg g^{-1} DW. This value represents the double of the DHA content in fingerlings muscles fed on IDP and LDP. Initial muscle's DHA/EPA ratio (1.09 ± 0.05) was too weak compared with those obtained at the end of experiment, ranged between 1.9 and 3.4 for the three feeding diets fingerling groups. The n-3 HUFA contents were of 15.1 ± 0.47, 6.6 ± 0.11 and 9.8 ± 1.75 mg g^{-1} DW respectively for MP, LDP and IDP; these values were lower that the initial one obtained before the beginning of the experiment.

Table 3. Growth and conversion performances of Dentex's fingerlings fed three different diets.

Food	MP[1]	IDP[2]	LDP[3]
Initial weight	5.87±0.29	5.94±0.38	6.22±0.34
Final weight	17.11±0.51[a]	32.08±1.11[b]	41.66±1.86[c]
SGR (%)	1.70±0.01[a]	2.40±0.02[b]	2.70±0.05[c]
FCR	1.80±0.02[a]	1.30±0.04[b]	1.50±0.01[c]

Mean ± SEM affected with different exposing letters (a, b and c) are significantly different (P < 0.05) according to Neuwman-Keuls test (n=2). SGR = specific growth rate=100* (ln final weight – ln initial weight)/no days. FCR = Food conversion rate= intake food/won weight
[1]MP: moist pellet
[2]IDP: imported dry pellet
[3]LDP: locally dry pellet

DISCUSSION

Up to now, there are few studies leading to determine the nutritional requirements of common dentex in on-growing phase aiming to have optimal and profitable growth for fish farms. First experiments used various foods (fresh food, semi-wet, commercial food, dry food and extruded food), (Riera et al., 1993; Efthimiou et al., 1994; Cardenete et al., 1997a; Tibaldi et al., 1996). Efthimiou et al., (1994) used dry pellet to feed gilt-head sea-bream and wet food to feed large size juveniles of *Dentex* of mean weight 2.4 g. After six weeks, the individual mean weight of juveniles fed on pellets is 16.12 ± 1.58 g, and 31.3 ± 1.45 g for the ones fed on wet-food. Conversion rates are respectively 1.16 and 0.77. Mortalities, due to the cannibalism are 48.4% for the pellet trial and 36.4% for the wet-food trials. These survival rates are lower than those we obtained for the three tested foods. In our conditions, survival rate was of 90% for the moist pellet, 82.5% for the imported pellet and 62.5% for the locally pellet for a period equivalent to ten weeks, but statistically there was no significant difference. During this experiment the three foods used are moist pellet, imported dry pellet and locally dry with lipids contents respectively of 26, 21 and 10%. The obtained conversion rates were respectively 1.8, 1.3 and 1.5. They are in limits to those cited by Rueda and Martinez (2001), Espinosa et al. (2003) and Ait Ali et al. (2006b).

In terms of fatty acids, the MP presented the highest level in DHA and EPA compared with the two other foods. On the other hand, the LDP presented a highest content in 18:2n-6c and 18:3n-3; n-3 HUFA content was very higher in MP (23.0 ± 0.32 mg g^{-1} DW) compared with the two other pellets. Cardenete et al. (1997d) investi-gated an experimental food for which composition is known, which they compare with a fresh food by using individuals of 60 g mean weight. They have concluded that food containing 57% of proteins and 14.4% of lipids gave comparable results to those obtained with a fresh food in term of survival and growth.

Results obtained during our work showed that, the IDP containing 21% of lipids and the LDP containing 10% of

Table 4. Fatty acids composition (mg g-1 DW) of Dentex fingerlings muscles. Data represent means±SEM

Fatty acid	Muscle1	Muscle (MP)	Muscle (IDP)	Muscle (LDP)
14:0	6.7±0.01^{a}	5.6±0.29^{b}	6.4±0.33^{c}	5.2±0.20^{d}
15:0	5.4±0.01	5.6±0.03	5.4±0.02	5.3±0.01
16:0	16.4±0.91^{a}	24.0±0.11^{b}	19.6±0.20^{c}	16.4±0.00^{a}
17:0	4.0±0.04^{a}	5.6±0.01^{b}	5.4±0.00^{c}	5.3±0.01^{c}
18:0	12.1±0.21^{a}	8.7±0.05^{b}	7.6±0.23^{c}	6.8±0.30^{d}
16:1	8.7±0.32^{a}	8.4±0.01^{b}	8.0±0.15^{c}	8.7±0.06^{a}
17:1	3.1±0.05^{a}	3.3±0.01^{b}	3.2±0.06^{b}	3.3±0.01^{b}
18:1n-9c	14.0±0.44^{a}	20.6±0.16^{b}	27.6±0.85^{c}	24.6±0.81^{d}
18:1n-7	9.8±0.37^{a}	3.9±0.04^{b}	3.5±0.13^{c}	3.3±0.04^{d}
20:1n-9	0.8±0.03^{a}	4.2±0.53^{b}	1.3±0.05^{c}	2.9±2.02^{d}
22:1n-11	nd	2.4±0.20^{a}	3.8±0.25^{b}	4.8±0.58^{c}
22:1n-9	0.2±0.01^{a}	0.7±0.07^{b}	0.6±0.05^{b}	0.7±0.04^{b}
24:1n-9	0.4±0.06^{a}	1.0±0.13^{b}	0.4±0.26^{c}	0.1±0.01^{d}
18:2n-6c	4.0±0.09^{a}	2.8±0.04^{b}	8.5±0.08^{c}	9.4±0.40^{d}
20:4n-6	nd	0.6±0.01^{a}	0.2±0.01^{b}	0.4±0.01^{c}
20:2n-6	0.3±0.01^{a}	0.3±0.00^{a}	0.1±0.01^{b}	0.5±0.03^{c}
18:3n-3	2.1±0.08^{a}	0.8±0.00^{b}	0.1±0.01^{c}	nd
20:3n-3	0.2±0.01	0.2±0.00	0.1±0.01	0.2±0.08
20:5n-3	4.3±0.23^{a}	3.2±0.08^{b}	1.7±0.01^{c}	2.9±0.34^{d}
22:5n-3	1.6±0.16^{a}	1.1±0.08^{b}	0.7±0.01^{c}	1.3±0.11^{d}
22:6n-3	4.7±0.68^{a}	10.8±0.61^{b}	4.2±0.04^{a}	5.6±0.82^{c}
DHA/EPA	1.09±0.05^{a}	3.38±0.10^{b}	2.47±0.04^{c}	1.93±0.05^{d}
n-3 HUFA	10.6±1.04^{a}	15.1±0.47^{b}	6.6±0.11^{c}	9.8±1.75^{d}

n-3 HUFA = N-3 HUFA >20:3n-3; nd = not detected. Muscle 1= Muscle of fingerlings at the beginning of experiment.
Means ± SEM having different letters (a, b and c) indicate that treatments are significantly different (P<0.05) (Neuwman-Keuls test) (n=2).

lipids gave both highest growth rates than MP. SGR obtained for IDP (2.4) and LDP (2.7) are much higher than MP (1.7). While these values are superior to those reported for this specie (0.6 - 1.3) (Tibaldi et al., 1996; Cardenete et al., 1997c) and also other species as sea bass (Metailler and Hollocou, 1991), sar (Abellan et al., 1994), gilt-head sea bream (Kentouri et al., 1994). However they are found to be inferior to those cited for common dentex by Company et al. (1999) and Efthimiou et al. (1994). Many experiences have demonstrated for various species of fishes, an increase in feeding lipid con-tents gave economical effect on proteins use (Takeda et al., 1975; Vergara et al., 1996). This effect was not observed in Japanese sea-bream (*Pagrus major*) (Takeuchi et al., 1991) . Skalli et al. (2004) note that sea bass juveniles fed low dietary (n-3) HUFA (0.2% DM of the diet) showed growth lower than (0.7) and further improvement of growth was seen by elevating the n-3 HUFA level up to 1.9%. Probably an excess of energy was not able to promote further protein sparing and significant better growth (Vergara et al., 1999). All the fishes show a low growth and a high food conversion ratio when they consume poor regimes in fatty acids. Generally, nutritional deficiencies in fatty acids result from a bad formulation of food or from the use of poor alive food in fatty acids. Inverse effects were observed with the MP

rich in lipids (26%), this result was comparable to those obtained in situation of an excessive proportion of feeding lipids leading to a negative effect on growth and on the food conversion rate of some fishes as rainbow trout (Yu and Sinnhuber, 1976; Takeuchi and Watanabe, 1999) and Salmo coho (Yu, 1979) and also common dentex. Regost et al. (2001) has found that turbot also showed a net deficit of growth when feeding diet rich in lipids. For this species it seems that food should not contain more than 11% of lipids; otherwise growth will be negatively affected. Several studies showed that fatty acids composition of tissues reflects that of the ingested lipids. For rainbow trout, contents in (n-3) polyunsaturated fatty acids were all the more important in the muscle as regimes contain increasing quantities of lipids brought provided by fish oil, rich in fatty acids (Castledine and Buckley, 1980; Kim et al., 1989). This relation between dietary fatty acids and muscular fatty acids was revealing by several authors for many marine species such as cod (Lie et al., 1992) or turbot (Bell et al., 1994). In our experiment the composition of the MP rich in EPA and DHA reflects the composition of the fingerling's muscle fed on this same food. On the other hand, it's noted a relative increase of DHA rate in the fingerling's muscle fed on the LDP resulting probably from a weak elongation-desaturation which can take place from C18: 3n- 3. Whether, the production of 22:6n-3 in fish involved 4 desaturation of 22:5n-3 or 6 desaturation of 24: 5n-3 with chain shortening of the resultant 24:6n- 3 to 22:6n-3 (Buzzi et al., 1996, 1997). An increase of dietary lipid levels generally results in an increase of fat accumulation and difference in body lipid composition may be due to the experiment duration and fishes size (Vergara et al., 1996). However, high rates of dietary lipids lead to a fattening of fish. The rate of lipids in the sea bass muscle can so pass from 7.1% of fresh material to 9.2% when the rate of lipids in the food passes from 13 to 29%. In the same way, there will be a peri-visceral fattening, a fattening of liver and a modification of the intestinal membranes leading to metabolic disturbances (Cahu et al., 2000).

Conclusion

This study showed the capacity of common dentex fingerlings, for the same content of proteins, to use lipids. The dietary quantity of lipids can be used on a rational way to make food economy and to have optimal survival and growth rates. High lipid contents gave inverse effects in growth.

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