Dietary lipid level affects growth performance and nutrient utilisation of Senegalese sole (*Solea senegalensis*) juveniles

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Over the last few years, several aspects of Senegalese sole (*Solea senegalensis*) culture have been developed and optimised but the dietary lipid level for optimal growth has never been determined. Hence, five isonitrogenous diets (56 % dietary protein) with increasing dietary lipid levels (4, 8, 12, 16 and 20 % DM) were fed to satiation to triplicate groups of twenty fish (mean initial weight 10 g). Fifteen tanks were randomly assigned one of the five diets. Feed was distributed using automatic feeders, and fish were fed over a 16-week period. At the end of the experiment the fish fed on diets containing the two lowest dietary lipid levels (4 and 8 %) showed a 3-fold body-weight increase with a significantly higher daily growth index than fish fed higher lipid levels (1·2 *v.* 0·8). Moreover, these fish displayed a significantly lower dry feed intake (12 g/kg per d) and feed conversion ratio (1·0) compared with fish fed higher lipids levels (16–19 g/kg per d; feed conversion ratio 2·0). Low dietary lipid levels (< 12 %) significantly improved nutrient retention and gain and hence growth, without major effects on whole-body composition. Despite the slight alteration in *n*-3 PUFA muscle content in the fish fed low-fat-diets, this fish fed low dietary lipid still remains a rich *n*-3 PUFA product and generally maintained its nutritional value. These results evidenced a low lipid tolerance of Senegalese sole juveniles and suggest a maximal dietary inclusion level of 8 % lipids for both optimal growth and nutrient utilisation without compromising flesh quality.

Lipid nutrition: Flatfish: Dietary lipid tolerance: Protein-sparing effect

The aquaculture industry has expanded in Europe over the last decades, but few marine fish species have contributed to this growth. The successful development of Southern European aquaculture has resulted in increased production of gilthead seabream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*) and turbot (*Psetta maxima*), leading to market saturation and reduced prices. For these reasons, great efforts have recently been devoted to find new candidate species, to improve diversity and to ensure sustainable development of the industry.

Senegalese sole (*Solea senegalensis*) is a promising flatfish species for marine farming, especially due to its high market value. Over the last few years significant advances have been accomplished on Senegalese sole weaning techniques and larvae feeding[1-3]. However, little is known about the nutritional needs of this flatfish species during its juvenile stage[4,5]. The protein requirement for maximum protein accretion in Senegalese sole has been estimated at 600 g/kg diet[5], but, so far, no studies concerning the optimal dietary lipid level have been carried out.

Dietary formulations in aquaculture tend to increase lipid content as a non-protein source for enhancing growth and sparing protein, and for reducing organic matter and N losses[6-10]. Nevertheless, in flatfish species positive results on protein sparing are limited and controversial. High dietary lipids enhanced growth and protein utilisation in plaice (*Pleuronectes platessa*)[11], whereas no beneficial effects were reported in halibut (*Hippoglossus hippoglossus*)[12-17] and Senegalese sole[4]. Moreover, turbot (*Psetta maxima* and *Scophtalmus maximus*) was negatively affected by dietary lipids[17,18].

The quantity, quality and body fat stores vary widely among fish species and mainly depend on dietary factors. In salmonids, fat deposition occurs mainly in the viscera and to a lesser extent in the muscle[19], whereas in flatfish species, such as turbot, the marginal body parts and subdermal fat constitute the most important lipid deposition sites[18,20]. Increasing the dietary lipid levels can alter body composition and slaughter quality, particularly through an increase in lipid deposition[11-12,16,19] affecting flesh organoleptic properties and final consumer's acceptance.

The aim of the present study was to determine the optimal dietary lipid level that can simultaneously improve growth

without compromising final flesh quality in Senegalese sole juveniles. The effects of increasing dietary lipid levels (4 to 20 %) on growth, feed efficiency, whole-body composition, nutrient and energy retention and tissue lipid deposition were evaluated in Senegalese sole juveniles over a 16-week period.

Materials and methods

Experimental diets

Five isonitrogenous (56 % DM) experimental diets were formulated to contain increasing lipid levels (4, 8, 12, 16 and 20 % DM; L4, L8, L12, L16 and L20, respectively). The dietary protein fraction was achieved by means of a variable blend of practical ingredients such as fishmeal, soyabean meal, maize gluten and wheat gluten, whereas increasing lipid levels were obtained by increasing fish oil inclusion.

Ingredients and proximate composition are presented in Table 1. Ingredients were finely ground, mixed and pelleted dry without steaming using a laboratory pelleting machine (C-300 model; California Pellet Mill, San Francisco, CA, USA) with a 2·0 mm die. The diets were dried at 37°C for 24 h and stored in a refrigerator (4 ± 1°C) until use.

Growth trial

Experiments were directed by trained scientists (following FELASA category C recommendations) and were conducted according to the European Economic Community animal experimentation guidelines directive of 24 November 1986 (86/609/EEC). The study was performed at the experimental facilities of CIIMAR (Porto, Portugal), with Senegalese sole (*Solea senegalensis*) juveniles supplied from a commercial fish farm (Alrogal, La Coruña, Spain). After arrival at the

Table 1. Ingredients, proximate composition and fatty acid composition (g/100 g fatty acids) of the experimental diets with different levels of dietary lipids

	Dietary treatments (%)				
	L4	L8	L12	L16	L20
Ingredients (%)					
Fishmeal LT	37	37	37	37	37
CPSP G	1·0	2·0	3·5	3·5	3·5
Squid meal	5	5	5	5	5
Soyabean meal 48	16·0	16·0	14·0	13·3	9·5
Maize gluten	12·5	12·5	12·0	12·0	9·0
Wheat meal	23·0	18·0	14·5	10·0	8·0
Wheat gluten	3·0	3·0	3·5	4·5	9·0
Gelatin	2	2	2	2	2
Fish oil	0	4·0	8·0	12·2	16·5
Choline chloride	0·1	0·1	0·1	0·1	0·1
Lutavit C35	0·03	0·03	0·03	0·03	0·03
Lutavit E50	0·05	0·05	0·05	0·05	0·05
Mineral and vitamin mix*†	0·25	0·25	0·25	0·25	0·25
Betaine	0·07	0·07	0·07	0·07	0·07
Proximate composition					
DM (%)	91·30	91·70	92·20	92·44	92·61
Ash (% DM)	8·48	8·44	8·30	7·85	7·82
Crude protein (% DM)	57·02	56·06	56·82	57·52	56·06
Crude fat (% DM)	4·07	8·40	13·67	17·35	22·50
Gross energy (kJ/g DM)	20·56	21·78	22·87	23·81	24·58
Fatty acid composition					
Σ SFA‡	24·66	24·06	23·93	23·09	23·59
Σ MUFA§	20·88	28·59	33·09	33·41	33·74
Σ PUFA‖	48·47	41·96	37·30	37·47	36·42
Σ *n*-3¶	25·24	25·43	24·81	24·83	23·62
DHA:EPA	1·13	1·15	1·15	1·17	1·16
EPA:ARA	13·12	13·70	13·54	13·49	13·19

LT, low temperature; CPSP G, fish soluble protein concentrate (hydrolysed fishmeal); ARA, arachidonic acid.

* Vitamins (per kg diet): vitamin A, 8000 IU (2·4 mg); vitamin D_3, 1700 IU (0·04 mg); vitamin K_3, 10 mg; vitamin B_{12}, 0·02 mg; vitamin B_1, 8 mg; vitamin B_2, 20 mg; vitamin B_6, 10 mg; folic acid, 6 mg; biotin, 0·7 mg; inositol, 300 mg; nicotinic acid, 70 mg; pantothenic acid, 30 mg; vitamin E (Lutavin E50), 300 mg; vitamin C (Lutavin C35), 500 mg; betaine (Betafin S1), 500 mg.

† Minerals (per kg diet): Mn (manganese oxide), 20 mg; I (potassium iodide), 1·5 mg; Cu (copper sulfate), 5 mg; Co (cobalt sulfate), 0·1 mg; Mg (magnesium sulfate), 500 mg; Zn (zinc oxide), 30 mg; Se (sodium selenite), 0·3 mg; Fe (iron sulfate), 60 mg; Ca (calcium carbonate), 2·15 g; dibasic calcium phosphate, 5 g; KCl, 1 g; NaCl, 0·4 g.

‡ Σ SFA = sum of 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0 and 24:0.

§ Σ MUFA = sum of 16:1, 18:1, 20:1 and 22:1.

‖ Σ PUFA = sum of 18:2*n*-6, 18:3*n*-6, 18:3*n*-3, 20:2*n*-6, 20:3*n*-3, 20:4*n*-6, 20:5*n*-3, 22:4*n*-6, 22:5*n*-3 and 22:6*n*-3.

¶ Σ *n*-3 = sum of 18:3*n*-3, 20:3*n*-3, 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3.

experimental unit fish were acclimatised to the new rearing facilities for 2 weeks. For each treatment, triplicate groups of twenty fish (average initial body weight 9·9 (SD 1·8) g) were distributed among fifteen fibreglass tanks (50 × 35 cm; initial fish density 1 kg/m^2). Each tank was supplied with filtered, heated (20 ± 1°C) seawater (30 ‰), at a flow rate of 2 litres per min. The most important physical and chemical parameters (temperature, dissolved O$_2$, salinity, pH and nitrogenous compounds) were monitored during the entire trial and maintained at levels within limits recommended for marine species. Fish were exposed to an artificial photoperiod of 12 h light–12 h dark. At the beginning and end of the experiment individual fish weights were recorded. Fish were fed by automatic feeders 24 h per d. The ration offered was daily adjusted based on the feed losses in each tank. Each day, all tanks were monitored to be certain that all feed was eaten and hence, no feed lost was registered. When some uneaten food remained in the bottom of the tank, the total amount of feed distributed each day was reduced by 10 %, until no feed losses were recorded. When no feed losses were observed the amount of food was maintained for 4 d and then augmented 10 % during all the experiment.

At the beginning of the experiment, a pooled sample of ten fish from the initial stock was sampled and stored at −20°C for subsequent whole-body composition analysis. Liver and viscera weights were also recorded. At the end of the trial, four fish from each tank were sampled for the same purpose. Liver, viscera, dorsal muscle and skin were removed from three fish per tank, frozen in liquid N$_2$ and stored at −80°C for lipid analysis. All fish sampled were killed by a sharp blow on the head.

Analytical methods

Whole fish from each tank were ground, pooled and moisture content was determined (105°C for 24 h). Fish and tissues were subsequently freeze-dried before further analysis. Feed and whole-body samples were analysed for ash by combustion in a muffle furnace (Nabertherm L9/11/B170; Bremen, Germany; 550°C for 6 h), crude protein (Kjeldatherm TR and Vapodest 40; Gerhardt, Königswinter, Germany; micro-Kjeldahl; N × 6·25) after acid digestion, lipid content by petroleum ether extraction using a Soxtherm Multistat/SX PC (Gerhardt, Königswinter, Germany; 40–60°C) and gross energy in an adiabatic bomb calorimeter (Werke C2000; IKA, Staufen, Germany).

The extraction of total lipids in diets and tissues was carried out following the Folch *et al.* [21] method with chloroform–methanol (2:1) and gravimetric determination. Liver, viscera, muscle and skin samples were analysed individually. Fatty acid methyl esters (FAME) were prepared by acid-catalysed transmethylation of total lipids using boron trifluoride methanol according to Shantha & Ackman[22] and were analysed in a Chrompack CP 9001 gas chromatograph (Middelburg, The Netherlands). The chromatograph was equipped with a CP-Sil 88 fused silica capillary column (Varian, Middelburg, The Netherlands; 50 m × 0·25 mm internal diameter, film thickness 0·2 μm). The gas carrier used was He (120 kPa) and the thermal gradient was 5° minimum at 140°C, 140°C to 220°C at 5°C per min and a constant temperature of 220°C during 15 min. Injector and detector temperatures

were 250°C and 270°C, respectively. FAME were identified by comparison with standard mixtures (FAME 37 and PUFA 3; Supelco, Bellefonte, PA, USA) and data analysed using CP-Maitre software (version 2.5; Chrompack International, Bergen op Zoom, The Netherlands).

Statistical analysis

Statistical analyses followed methods outlined by Zar[23]. All data were tested for homogeneity of variances by Levene's tests, and then submitted to a one-way ANOVA with the SPSS 15.0 package (SAS Institute, Inc., Cary, NC, USA). When data did not meet the assumptions of ANOVA, the non-parametric ANOVA equivalent (Kruskal–Wallis test) was performed. When these tests showed significance, individual means were compared using Tukey or Mann–Whitney tests. Significant differences were considered when $P \le 0\cdot05$.

Results

At the end of the trial (112 d) average body weight showed a threefold increase for all dietary treatments. Final body weight, daily growth index, feed conversion ratio, protein efficiency ratio and nutrient intake are summarised in Table 2. High-fat (HF) diets (L12, L16 and L20) resulted in significant lower final body weight (29–33 g) when compared with the two low-fat (LF) diets (L4 and L8) (about 43 g). Daily growth index varied between 0·8 and 1·2 and was significantly affected by dietary lipid levels. Fish fed the HF diets showed significantly lower growth rates compared with those fed the LF diets.

Feed conversion ratio values ranged from 1·0 (L4) to 2·2 (L20) with fish fed L4 and L8 showing the best feed conversion ratio and fish fed L20 being significantly less efficient than all the others. Protein efficiency ratio varied from 0·8 to 1·7 with the highest and lowest values being, again, found in fish fed the LF (L4 and L8) and L20 diets, respectively. DM intake varied significantly among treatments, increasing with the lipid dietary level, and hence affecting significantly the intake of all other nutrients. The LF diets displayed the lowest nutrient intake, whereas fish fed L20 showed an opposite trend.

At the end of the trial, whole-body moisture, energy and ash contents were similar among treatments (Table 3). However, fish fed L16 or L20 displayed significantly lower whole-body protein than fish fed L8, and higher lipid content than fish fed L4. Nutrient and energy retention (Table 3) were significantly affected by the inclusion of dietary fat. Fish fed the LF diets displayed higher protein, lipid and energy retention than those fed the HF diets. A similar trend was observed for protein, but not for lipid gain.

The hepatosomatic index varied from 0·8 to 1 % and liver lipid content ranged from 8·0 to 10·1 %. The hepatosomatic index was significantly higher in fish fed L16 than in those fed the L8 and L12 diets. The viscerosomatic index varied from 2·1 to 3·6 % and was significantly higher in fish fed the HF diets than in those fed the LF ones (Table 4).

Senegalese sole muscle, liver and viscera lipid content are reported in Table 4. Skin and intestine were the preferential tissues for lipid deposition. Dietary lipid level significantly affected viscera and skin, but not muscle or liver lipid

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Table 2. Effect of different dietary lipids in Senegalese sole (*Solea senegalensis*) growth and intake after 112 d

(Mean values and standard deviations (*n* 3))

Diet...	L4		L8		L12		L16		L20	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Growth										
Initial body weight (g)	9·89	0·01	9·96	0·06	9·97	0·02	9·91	0·03	9·92	0·06
Final body weight (g)	43·66[a]	6·54	42·54[a]	1·51	33·02[b]	0·56	32·57[b]	2·61	28·54[b]	2·56
DGI*	1·22[a]	0·16	1·20[a]	0·03	0·94[b]	0·02	0·93[b]	0·07	0·81[b]	0·08
FCR†	1·04[c]	0·05	1·15[c]	0·03	1·66[b]	0·09	1·68[b]	0·06	2·16[a]	0·18
PER‡	1·70[a]	0·08	1·56[a]	0·04	1·06[b]	0·06	1·04[b]	0·04	0·83[c]	0·07
Intake§										
DM (g/kg ABW per d)	11·58[c]	0·30	12·70[c]	0·42	15·86[b]	1·06	15·96[b]	0·43	18·53[a]	0·64
Protein (g/kg ABW per d)	6·61[c]	0·17	7·12[c]	0·24	9·01[b]	0·60	9·18[b]	0·25	10·39[a]	0·36
Lipids (g/kg ABW per d)	0·47[e]	0·01	1·07[d]	0·04	2·17[c]	0·14	2·77[b]	0·07	4·17[a]	0·14
Energy (kJ/kg ABW per d)	2·38[c]	0·06	2·77[c]	0·09	3·63[b]	0·24	3·80[b]	0·10	4·55[a]	0·15

DGI, daily growth index; FCR, feed conversion ratio; PER, protein efficiency ratio; ABW, average body weight.
[a–e] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
* DGI = $100 \times$ ((final body weight)$^{1/3}$ − (initial body weight)$^{1/3}$)/days.
† FCR = dry feed intake/weight gain.
‡ PER = weight gain/crude protein intake.
§ Nutrient intake = nutrient intake/average body weight ((initial body weight + final body weight)/2)/days.

contents. Viscera fat content ranged from 3·3 to 7·7 % wet weight whereas skin fat values varied from 1·7 to 2·3 % wet weight, and significant differences were observed among treatments. Fish fed the L16 and L20 diets presented significantly higher viscera and skin lipid contents than those fed with L4. Although muscle total lipids did not vary among dietary treatments, the muscle of fish fed the L4 diet exhibited significantly higher percentages of SFA when compared with the muscle of fish fed the other diets (26 *v.* about 23–24 %). A totally different trend was observed in MUFA, with values

ranging from 22 to 29 %, and L12 exhibiting the highest content. Moreover, neither PUFA nor DHA:EPA and EPA:arachidonic acid ratios were affected by the dietary lipid levels, whereas *n*-3 fatty acids were highest in fish fed L20.

Discussion

At the end of the growth trial, final body weight was about triplicate in all treatments, and dietary lipid levels higher than 8 % were shown to significantly depress growth performance

Table 3. Whole-body composition, nutrient retention and gain of Senegalese sole (*Solea senegalensis*) fed increasing dietary lipid levels for 112 d*

(Mean values and standard deviations (*n* 3))

Dietary treatment...	L4		L8		L12		L16		L20	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Whole-body composition										
Moisture (% wet weight)	76·43	0·80	75·07	1·28	76·25	0·63	76·56	0·66	75·65	0·27
Ash (% wet weight)	1·79	0·19	1·73	0·27	1·89	0·41	1·63	0·16	1·59	0·08
Protein (% wet weight)	17·69[a,b]	0·35	18·24[a]	1·04	16·93[a,b]	0·47	15·98[b]	0·54	16·18[b]	0·42
Lipids (% wet weight)	3·72[b]	0·22	5·02[a,b]	0·44	5·14[a,b]	0·10	5·69[a]	0·84	5·84[a]	0·66
Energy (kJ/g wet weight)	5·61	0·13	6·17	0·33	5·82	0·03	5·85	0·38	5·98	0·11
Retention (% feed intake)†										
DM	22·57[a]	0·12	21·92[a]	1·36	14·19[b]	0·21	13·71[b]	0·63	11·34[b]	1·12
Protein	30·23[a]	0·61	28·89[a]	2·07	17·87[b]	0·25	15·97[b,c]	0·42	12·99[c]	1·63
Lipids	82·47[a]	1·84	53·57[b]	4·67	23·9[c]	1·95	21·24[c]	4·19	13·47[c]	1·8
Energy	26·14[a]	0·50	25·14[a]	1·43	15·39[b]	0·72	14·65[b,c]	1·32	11·50[c]	1·04
Gain‡										
DM (g/kg ABW per d)	2·61[a,b]	0·08	2·78[a]	0·16	2·25[b,c]	0·12	2·19[b,c]	0·16	2·10[c]	0·18
Protein (g/kg ABW per d)	2·00[a]	0·09	2·06[a]	0·13	1·61[b]	0·08	1·47[b]	0·07	1·35[b]	0·15
Lipids (g/kg ABW per d)	0·39	0·001	0·57	0·07	0·52	0·01	0·59	0·13	0·56	0·09
Energy (kJ/kg ABW per d)	0·62[a,b]	0·03	0·70[a]	0·05	0·56[a,b]	0·01	0·56[a,b]	0·06	0·52[b]	0·05

ABW, average body weight.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
* Initial body composition was: moisture, 75·65 % wet weight; ash, 2·49 % wet weight; protein, 17·22 % wet weight; lipids, 4·53 % wet weight; energy, 5·81 kJ/g wet weight.
† Retention = $100 \times$ (final body weight × final carcass nutrient content − initial body weight × initial carcass nutrient content)/nutrient intake.
‡ Nutrient gain = (nutrient in final carcass − nutrient in initial carcass)/average body weight/days.

Table 4. Somatic indexes (%), tissue total lipids and muscle fatty acids classes of Senegalese sole (*Solea senegalensis*) fed increasing dietary lipid levels for 112 d

(Mean values and standard deviations (*n* 6, except for somatic indexes, where *n* 9))

Dietary treatment...	L4 Mean	L4 SD	L8 Mean	L8 SD	L12 Mean	L12 SD	L16 Mean	L16 SD	L20 Mean	L20 SD
HSI*	0.83[a,b]	0.22	0.73[b]	0.16	0.73[b]	0.10	1.00[a]	0.29	0.86[a,b]	0.12
VSI†	1.98[b]	0.41	2.11[b]	0.31	2.10[b]	0.31	3.60[a]	1.41	3.04[a]	0.39
Tissue total lipids (% wet weight)										
Liver	9.38	2.14	8.81	0.98	8.02	1.98	10.10	1.81	9.09	1.26
Viscera	3.33[b]	0.39	3.17[b]	0.17	3.96[a,b]	0.78	6.07[a]	1.35	7.68[a]	1.02
Skin	1.72[b]	0.21	2.16[a,b]	0.26	2.15[a,b]	0.39	2.33[a]	0.41	2.24[a]	0.18
Muscle	2.06	0.50	2.74	1.24	3.05	1.38	2.20	0.63	2.35	0.54
Muscle fatty acid composition (g/100 g fatty acids)										
Σ SFA‡	26.09[a]	0.76	23.82[b]	1.78	23.53[b]	0.42	24.13[b]	0.71	23.14[b]	0.95
Σ MUFA§	22.17[b]	2.63	26.57[a]	2.94	29.37[a]	2.81	26.93[a]	1.48	27.67[a]	1.65
Σ PUFA‖	41.39	1.58	38.97	2.47	38.62	1.81	39.30	1.04	39.58	0.96
Σ *n*-3¶	25.66[b]	1.84	26.01[b]	2.02	26.08[b]	2.11	28.14[a,b]	1.52	29.31[a]	1.39
DHA:EPA	4.60	0.73	4.22	0.82	3.52	0.61	4.54	0.56	4.23	0.54
EPA:ARA	4.10	1.33	4.08	0.68	5.67	1.29	3.78	0.69	4.31	1.37

HSI, hepatosomatic index; VSI, viscerosomatic index; ARA, arachidonic acid.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
* HSI = 100 × (liver weight/body weight) (%).
† VSI = 100 × (viscera weight/body weight) (%).
‡ Σ SFA = sum of 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0 and 24:0.
§ Σ MUFA = sum of 16:1, 18:1, 20:1 and 22:1.
‖ Σ PUFA = sum of 18:2*n*-6, 18:3*n*-6, 18:3*n*-3, 20:2*n*-6, 20:3*n*-3, 20:4*n*-6, 20:5*n*-3, 22:4*n*-6, 22:5*n*-3 and 22:6*n*-3.
¶ Σ *n*-3 = sum of 18:3*n*-3, 20:3*n*-3, 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3.

(final body weight and daily growth index). In spite of the dietary protein fraction being achieved by means of a variable blend of practical ingredients such as fishmeal, soyabean meal, maize gluten and wheat gluten, the higher inclusion of vegetable protein sources in the LF diets improved growth and nutrient retention, suggesting a good acceptance of plant sources by solea juveniles. Therefore, the observed differences in growth performance among treatments seem to be mainly due to the dietary lipid levels. This is in agreement with previous studies in turbot that reported a decline in growth rate with diets containing increasing dietary fat levels either in juvenile[17] or commercial-sized fish[18]. A growth depression was also observed when halibut juveniles were fed a diet containing 30 % lipid and a low level of carbohydrates[15]. Nevertheless, increasing dietary fat levels from 11 % to 21 % in Senegalese sole juveniles during 90 d[4] or from 8 to 28 % in different Atlantic halibut size classes did not seem to affect growth performance[12–14,16,24]. Unlike salmonids or marine fish species where a protein-sparing effect has been clearly demonstrated[6–8,10,25], no beneficial effects on overall growth performance were observed in Senegalese sole juveniles fed high dietary lipid levels.

The high nutrient intake (12–19 g/kg average body weight per d) and low feed conversion ratio (1–2) values observed in this trial reflect the good acceptability of the diets by sole. Dias *et al.*[4] reported similar growth performance of Senegalese sole juveniles fed either 11 or 21 % lipids for 90 d, although the registered feed conversion ratio (2·4–3) was clearly inferior to that observed in the present stsudy. The 11 % fat included in those diets did not induce significant differences among dietary treatments, although a slight decrease in weight gain was also noticed in fish fed 21 % lipids. This trend could have resulted in significant differences

among dietary treatments if a longer trial duration had been carried out. The high feed conversion ratio values could be related to distinct feeding protocols. In its natural habitat sole remains inactive and buried in the sand the whole day, and carries out all its activity, particularly feeding, during the night[26]. Hence, instead of feeding animals 18 h per d as adopted by Dias *et al.*[4] it seems advisable to feed fish 24 h per d to achieve maximal growth performance and improve feed conversion. In the present trial, the dietary lipid levels strongly affected both intake and nutrient utilisation, the LF diets being the most efficient in displaying the lowest nutrient intake but best feed conversion ratio and protein efficiency ratio. In fact, the increase in the input of non-protein energy substrates has a deleterious effect on fish growth performance which seems to be related to their poor utilisation by Senegalese sole.

Senegalese sole is considered a lean fish with low whole-body fat contents (< 6 %)[4,5]. In the present study whole-body lipid content varied from 4 to 6 %, with the L4 diet inducing significantly lower whole-body fat content than L16 and L20 diets. Similarly, in turbot such an effect has been observed when dietary fat levels increased from 10 to 25 %[17]. Dias *et al.*[4] has also reported an increased fat content in sole fed HF diets (10 *v.* 21 %), but only when associated with low-digestible carbohydrates. Increased whole-body fat content due to an increase in dietary fat has been widely reported in salmonids[8], or other marine teleosts, such as seabass[27], gilthead sea bream[28] or Atlantic halibut[12,13,16], although all these species display significantly higher whole-body fat contents compared with sole. Increasing dietary lipid levels in Senegalese sole resulted in decreased lipid retention, but without any significant effects on fat gain. On the other hand, both protein gain and retention

were improved by the decrease in dietary lipid. In contrast, Dias *et al.* [4] observed that Senegalese sole fed diets with 20 % dietary lipids showed similar protein gain but a significantly higher fat gain ($P < 0.05$) when compared with fish fed diets with 10 % lipids. Similarly, in other flatfishes such as turbot[18] and Atlantic halibut[16], protein utilisation was not improved by an increase in dietary lipids. However, other studies with Atlantic halibut reported a protein-sparing effect with increasing dietary lipids[12,24], though the diets were not isonitrogenous. In species such as salmon[8], trout[10], gilthead seabream[28] and European seabass[25], protein retention may be improved by partly replacing dietary protein by lipids, but in flatfish species this protein sparing seems to be controversial. In Senegalese sole such a wide dietary lipid range (4–20 %) has never been evaluated before, but the obtained results suggest a poor utilisation of the lipid fraction that warrants further investigation. The determination of nutrients' apparent digestibility coefficients could help clarifying this point. Several attempts for faeces collection have been carried out; nevertheless neither decantation methods nor fish stripping have been proven to be feasible for this species, due to the physical properties of the faeces and the morphology of the intestine. The dissection of the intestine seems to be the only way to recuperate faeces, but a great number of juveniles or larger-sized fish are required. Moreover, in the present study, the lack of a protein-sparing effect by dietary lipid might also be related to the high protein level of the experimental diets. Dias *et al.* [25] reported that, in sea bass, an increase in lipid level from 10 to 18 % only was beneficial at low protein levels (40 %). This suggests the need to optimise the dietary protein:lipid ratio to the metabolic needs of Senegalese sole juveniles.

Liver lipid content ranged from 8·0 to 10·1 % of liver wet weight, and was unaffected by dietary lipid level. Overall, liver lipids contribute to 0·1 % of final body weight and 1·7 % of total lipid content. Higher liver lipid contents were reported by Dias *et al.* [4] in this species and in species such as turbot[18] and Atlantic halibut[16]. Moreover, Dias *et al.* [4] observed that elevated levels of dietary lipids in Senegalese sole increased liver lipid content and depressed the lipogenic enzymic activity. Different results were reported in turbot, where the absence of any change in hepatic lipogenic enzymes was associated with a low lipid deposition and relative absence of response to high dietary lipid levels. In the present study, significant differences were found for hepatosomatic index among dietary treatments, but there was no clear association with dietary lipid level since L4 and L20 were similar. Hepatosomatic index values reported are in accordance with those found by Dias *et al.* [4] in Senegalese sole but lower than other flatfish species such as turbot[18] and Atlantic halibut[12,16,24] and other marine teleosts such as European seabass[25] and gilthead seabream[29].

Both the viscerosomatic index and viscera total lipids of Senegalese sole increased with dietary lipid levels but were generally lower than values previously found in other teleosts including flatfish species[4,18,29,30]. Lipid content of the viscera represents 0·1–0·2 % of body weight and 4 % of the whole-body lipids. Despite the higher values observed in other marine species, it is clear that Senegalese sole tends to accumulate part of the fat in the viscera. Moreover, fish fed HF diets exhibit a significantly higher viscerosomatic index, essentially due to increased fat deposition in the viscera. This is in general accord with earlier observations by Aksnes *et al.* [12] that reported similar results in halibut with increasing dietary lipids. Nevertheless, lower values of fat in the viscera (0·2–0·8 %) were previously reported in Senegalese sole[4] and could not induce viscerosomatic index differences among treatments. In halibut, it was observed that a high dietary lipid level (13 to 32 %) decreased slaughter quality[12], but that was not found in turbot[18]. In the present study, viscera total lipids decreased with the LF diets, contributing to a significant decrease of whole-body lipid composition but an increase in the protein content. These results suggest a poor utilisation of dietary lipids and the potential value of such LF diets in improving Senegalese sole growth potential.

The content of total lipids of dorsal muscle ranged from 2·1 to 3·1 % on a fresh-weight basis, representing 1·3 % of whole-body weight and 27 % of total whole-body lipids. Nevertheless, contrarily to the viscera, muscle total lipids were not affected by increasing dietary lipid levels. These results are in agreement with earlier observations in flatfish species such as halibut[13,16], turbot[18] and Senegalese sole[4], and suggest that other tissues such as skin and subdermal adipose tissue or other carcass fraction (fins, brain, bones) might be important fat-storage sites. In the present study, Senegalese sole subdermal fat only represented about 0·2 % of whole-body weight and 4 % of total body lipids, showing that lipids are probably being accumulated under the skin like in turbot[18] and between the dorsal and ventral fins and around the belly flap like in halibut[31].

Besides being energy-providing nutrients, dietary lipids are important in marine fish as a source of essential fatty acids, especially $20:5n\text{-}3$ (EPA), $2220:6n\text{-}3$ (DHA) and $2020:4n\text{-}6$ (arachidonic acid)[32]. The fatty acid compositions of tissue lipids in fish are well known to be influenced by the fatty acid composition of dietary lipids[32]. This was the case in the present study, where a linear correlation existed between the concentrations of given fatty acids in dietary lipids and their concentrations in flesh total lipids. Taking into account that no significant differences were found in muscle total lipids among dietary treatments, fish fed the L4 diet effectively contained lower levels of $n\text{-}3$ fatty acids if compared with L20-fed ones. Additionally, the L4 diet induced a lower MUFA content, probably leading to the mobilisation of the $n\text{-}3$ fatty acids for energetic proposes. Nevertheless, the L20 diet reduced growth and did not improve nutrient utilisation in this species and hence does not seem to be suitable for Senegalese sole juveniles. Despite the slight alteration in $n\text{-}3$ muscle content in the fish fed the LF diets, this fish still remains an $n\text{-}3$-rich product contributing to the human health benefits associated with fish consumption, especially regarding DHA and EPA[33,34]. In fact, even at dietary lipid levels as low as 4 %, Senegalese sole DHA:EPA and EPA:arachidonic acid ratios remained unchanged and hence muscle maintained its nutritional value. Moreover, in southern European countries this fish is generally consumed grilled without removing the skin. As the skin is one of the preferential organs for fat deposition in flatfish, it could be suspected that some lipid migration of the fat deposited in the subdermal adipose tissue could occur during the cooking process, increasing even further the nutritional value of the flesh.

In conclusion, the results of the present study evidenced a low lipid tolerance by Senegalese sole juveniles and suggest

a maximal dietary inclusion level of 8 % lipids for both optimal growth and nutrient utilisation. Future studies are required to ascertain whether the absence of a clear protein-sparing effect by dietary lipids holds up at lower dietary protein levels or in large-sized fish. Concerning the lipid metabolism of this flatfish, it would be worthwhile investigating which are the mechanisms responsible for the apparent poor utilisation of the dietary lipids. A better understanding of the metabolic changes induced by the dietary protein:lipid ratio should allow adjustment of the diet composition to the metabolic capacities of Senegalese sole juveniles.

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