Available online at www.sciencedirect.com
SCIENCE DIRECT
Aquaculture 252 (2006) 85–91
Aquaculture
www.elsevier.com/locate/aqua-online

Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles

L.M.P. Valente [a,b,*], A. Gouveia [a,c], P. Rema [a,d], J. Matos [a], E.F. Gomes [a,b], I.S. Pinto [a,e]

[a] *CIIMAR–Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas, 177, 4050-123, Porto, Portugal*
[b] *ICBAS–Instituto Ciências Biomédicas 'Abel Salazar', Universidade do Porto, 4099-003, Porto, Portugal*
[c] *Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, Pr. Gomes Teixeira, 4099-002, Porto, Portugal*
[d] *Departamento de Zootécnia, Universidade de Trás-os-Montes e Alto Douro, 5001, Vila Real, Portugal*
[e] *Departamento de Botânica, Faculdade de Ciências, Universidade do Porto, Rua Campo Alegre 1191, 4150-181, Porto, Portugal*

Received 6 April 2005; accepted 18 November 2005

Abstract

The aim of this study was to evaluate the inclusion of three seaweeds *Gracilaria bursa-pastoris* (GP), *Ulva rigida* (UR) and *Gracilaria cornea* (GC) as dietary ingredients on the performance, nutrient utilisation and body composition of European sea bass juveniles. Six experimental diets were formulated to replace 5% (GP-5, UR-5, and GC-5 Diets) and 10% (GP-10, UR-10 and GC-10 Diets) fish protein hydrolysate (CPSP) by each of the three seaweeds. A control diet was used, without inclusion of any seaweed. Diets were fed to duplicate groups of 25 European sea bass (*Dicentrarchus labrax*) juveniles (IBW = 4.7 g) for 10 weeks. Growth performance was only significantly reduced ($P<0.05$) in fish fed the GC-10 diet, whereas the feed conversion ratio increased significantly in those fish. The apparent digestibility coefficients of dry matter and lipid were significantly lower in fish fed diet GC-10 relative to those fed the control diet. Carcass composition was similar among treatments, although fish fed GC-10 exhibited significantly higher ash content.

The results obtained in this study suggest that the inclusion of *G. bursa-pastoris* (GP) and *U. rigida* (UR), up to 10%, can be considered as very interesting ingredients in diets for sea bass juveniles, as no negative consequences on growth performance, nutrient utilization or body composition were observed. On the other hand, the inclusion of *G. cornea* (GC) should be limited to 5% of the diet.

Keywords: European sea bass; Alternative feed ingredients; Seaweeds; *Gracilaria bursa-pastoris*; *Ulva rigida*; *Gracilaria cornea*; Fish nutrition

1. Introduction

The long-term sustainability of aquaculture may be threatened by its present over-dependence on fish meal and fish oil (FAO, 2002). Moreover, fish feeding represents over 50% of operating costs in intensive aquaculture, with protein being the most expensive dietary

* Corresponding author. CIIMAR–Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas, 177, 4050-123, Porto, Portugal. Tel.: +351 223401800; fax: +351 22 339 06 08.
E-mail address: lvalente@icbas.up.pt (L.M.P. Valente).

0044-8486/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2005.11.052

source (Lovell, 2002). Therefore an intensive effort during these last decades has been made in order to evaluate the potential of alternative protein sources in aquafeeds (Alexis, 1997). Although several studies were conducted to evaluate the replacement of fish meal by plant ingredients in diets of European sea bass (Dias, 1999; Gouveia and Davies, 1998, 2000; Da Silva and Oliva-Teles, 1998; Kaushik et al., 2004), data about the potential use of seaweeds in fish diets is scarce (Appler, 1985; Nakagawa et al., 1987; Hashim and Mat Saat, 1992; El-Sayed, 1994, 1999; Davies et al., 1997; Wahbeh, 1997).

At present, seaweeds with elevated protein content and production rates are receiving increasingly attention as novel feeds with potential nutritional benefits (Buschmann et al., 2001; Rupérez and Saura-Calixto, 2001) and as possible ingredient in fish diets (Appler, 1985; El-Sayed, 1994; Davies et al., 1997; Wahbeh, 1997). Production of seaweeds has been increasing in the last decades but cultivation on a commercial scale remains restricted to a few species (Buschmann et al., 2001; Nagler et al., 2003). Still, nitrogen-enriched conditions like the effluents of fish farms, where seaweeds are used as biofilters, can increase their protein content (Lahaye et al., 1995; Pinchetti et al., 1998). Apart of their potential nutritional value as protein substitutes, algae may also give an important contribute in fish diets as lipid sources (Nakagawa et al., 1987), and binding (Hashim and Mat Saat, 1992) or colouring agents (Sommer et al., 1992; Gouveia et al., 2003).

The aim of the present study was to evaluate the use of three marine seaweeds, *Gracilaria bursa-pastoris* (GP), *Ulva rigida* (UR) and *Gracilaria cornea* var. red (GC), cultivated in effluents of fish farms, as dietary ingredients in partial substitution of fish protein hydrolysate (CPSP), on growth performance, nutrient utilisation and body composition of European sea bass (*Dicentrarchus labrax*) juveniles.

2. Material and methods

2.1. Experimental animals and trial conditions

The feeding trial was conducted at the Fish Culture Experimental Unit of University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal. Juveniles of European sea bass (*D. labrax*) were obtained at a commercial fish farm. After arrival at the experimental unit they were conditioned in two seawater tanks (1000-L capacity each) and acclimated to the local conditions for a period of 8 days. During this adaptation period, fish were fed a commercial diet supplied by Sorgal.

Fourteen homogeneous groups of 25 fish (initial mean body weight of 4.7 g) were then randomly distributed among 14 fiberglass tanks (55-L water capacity), in a recirculation water system. Each tank was supplied with filtered, heated (21 ± 1 °C) saltwater (34‰), at a flow rate of 2 L min^{-1}. 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 described as optimum for the species. Fish were exposed to an artificial photoperiod of 12-h light.

2.2. Feed and feeding

Six experimental diets were formulated to substitute 5% and 10% of fish protein hydrolysate (CPSP) by one of three seaweeds: *G. bursa-pastoris* (GP-5 and GP-10), *U. rigida* (UR-5 and UR-10) and *G. cornea* (GC-5 and GC-10). The proximate composition of the seaweeds is presented in Table 1. A control diet was used, without inclusion of any seaweed. The seaweeds used in this study are species currently found in the Portuguese coast and produced as a by-product of a fish farm effluent. They were sun-dried and grounded before being added to the dietary mixture at the expense of fish protein hydrolysate (CPSP). Feed ingredients and diet composition are summarized in Table 2.

All ingredients were grounded, mixed (in a horizontal helix ribbon mixer) and dry pelleted through a 2.4-mm die at 50 °C (CPM, C-300 model). Diets were subsequently stored at 5 °C. Every experimental diet was randomly assigned to two different tanks. Fish were fed twice daily until visual satiety for 10 weeks. Fish weight and fish consumption were registered every 3 weeks. A pooled sample of twelve fish from the initial group and six fish per tank at the end of the trial were sacrificed and frozen for subsequent carcass analysis.

2.3. Digestibility trial

The apparent digestibility (ADC) of the dietary components was measured after incorporation of 1 % of chromic oxide in each diet and using a specially

Table 1
Chemical composition of the dried seaweeds: *Gracilaria bursa-pastoris* (GP), *Ulva rigida* (UR) and *Gracilaria cornea* (GC)

	GP	UR	GC
Moisture (%)	8.3	11.9	10.0
Crude protein (% DM)	30.2	29.5	11.0
Crude lipid (% DM)	0.9	1.4	0.7

Table 2
Ingredients and chemical composition of the experimental diets

	Diet						
	Control	GP-5	GP-10	UR-5	UR-10	GC-5	GC-10
Ingredients (g kg^{-1})							
Fish meal (LT94)	610	600	590	600	590	600	590
CPSP [a]	100	50	–	50	–	50	–
Aquatex [b]	200	200	200	200	200	200	200
Gracilaria bursa-pastoris	–	50	100	–	–	–	–
Ulva rigida	–	–	–	50	100	–	–
Gracilaria cornea	–	–	–	–	–	50	100
Cod liver oil	30	40	50	40	50	40	50
Vitamin and mineral premix [c]	40	40	40	40	40	40	40
Binder [d]	20	20	20	20	20	20	20
Chromic oxide (%) [e]	1	1	1	1	1	1	1
Chemical composition							
Moisture (%)	9.1	8.8	9.1	9.1	9.3	9.0	8.7
Crude protein (% DM)	60.8	57.8	55.5	55.7	54.2	56.9	52.2
Crude lipid (% DM)	13.6	13.4	12.8	13.3	13.1	13.5	13.1
Ash (% DM)	12.0	12.7	13.2	13.3	14.6	15.3	18.7

[a] Soluble fish protein hydrolysate (72 CP; Sopropêche, France).
[b] Extruded dehulled pea seed meal (23 CP; Sotexpro, France).
[c] Per kg of mixture: vitamin A, 180 mg; vitamin D_3, 3 mg; vitamin E, 2.500 mg; vitamin K, 300 mg; vitamin B_{12}, 1 mg; vitamin B_3, 1.000 mg; vitamin B_5, 1.250 mg; folic acid, 75 mg; biotin, 15 mg; vitamin C, 2.500 mg; betaine, 25.000 mg; inositol, 15.000 mg; Co, 20 mg; Cu, 150 mg; Fe, 2.000 mg; F, 50 mg; I, 30 mg; Mg, 25.000 mg; Mn, 1.000 mg; Se, 15 mg; Zn 1.500 mg.
[d] Lignino sulphate.
[e] Incorporated only in diets for the digestibility trial.

constructed digestibility system according to the Guelph System protocol (Cho et al., 1982). Seven homogenous groups of 25 sea bass (mean body weight of 14 g) were stocked in each 55-L tank and adapted to the new conditions for 15 days. The experimental fish were subjected to a 12-h light/12-h dark photoperiod regime provided by artificial illumination, and water temperature was maintained at 21±1 °C.

The diets were randomly assigned by the tanks, being the experiment divided into three periods of 12 days, for replication of results (n=3). Each replicate was carried out in a different tank to reduce any tank effect. Before changing diets, the fish were fasted for 24 h and weighed. The amount of feed fed was then adjusted to meet the fixed feeding level adopted for the study. The first 2 days of each period were used for acclimation to the feed and no faeces were collected. This time period was deemed sufficient for the fish to achieve complete evacuation of previous meals.

Fish were fed 2% body weight once daily at 10.00 a.m. and the estimated amount of feed was carefully given to minimize uneaten feed. Approximately 30 min after sea bass consumed their meal any uneaten food and faeces were removed from the system. Before the fish were fed each morning, the faeces were collected and centrifuged at 3000×g for 10 min and stored for further analysis. The apparent digestibility coefficients (ADC) were calculated according to Maynard and Loosli (1969).

2.4. Analytical methods

Frozen whole body pooled samples and faeces were freeze-dried before analysis. Feed, whole body and faeces samples were analysed for dry matter (DM, 24 h at 100±1 °C), ash (15 h at 600 °C), crude protein (N×6.25, Kjeldahl method) and fat content (petroleum ether 40–60 °C extraction in a Soxlet apparatus); chromium oxide of diets and faeces was measured after acidic digestion according to Furukawa and Tsukahara (1966).

2.5. Statistical analysis

Statistical analyses followed methods outlined by Zar (1996). Data were tested for homogeneity of variance using Bartlett's test, and then submitted to a one-way ANOVA with the Statistics 6.0 for Windows package. When F values showed significance, individual means were compared using Tukey's honest significant

Table 3
Growth performance and feed utilization in European sea bass juveniles fed the experimental diets for 10 weeks

	Diet						
	Control	GP-5	GP-10	UR-5	UR-10	GC-5	GC-10
Initial body weight (g)	4.68±0.03[a]	4.67±0.04[a]	4.65±0.01[a]	4.66±0.03[a]	4.65±0.01[a]	4.66±0.00[a]	4.67±0.04[a]
Final body weight (g)	14.10±0.17[a]	14.25±1.14[a]	15.43±0.95[a]	13.09±0.06[a,b]	12.76±0.98[a,b]	13.08±0.32[a,b]	10.38±0.76[b]
Daily weight gain (% IBW)	2.93±0.08[a]	2.98±0.32[a]	3.37±0.28[a]	2.63±0.01[a,b]	2.54±0.32[a,b]	2.63±0.10[a,b]	1.78±0.27[b]
DGI	1.13±0.02[a]	1.14±0.09[a]	1.24±0.08[a]	1.04±0.000[a]	1.01±0.09[a,b]	1.04±0.03[a]	0.77±0.09[b]
FCR	1.51±0.002[a]	1.56±0.14[a]	1.48±0.17[a]	1.69±0.05[a,b]	1.80±0.23[a,b]	1.74±0.04[a,b]	2.31±0.37[b]
PER	1.09±0.002[a]	1.11±0.10[a]	1.22±1.14[a]	1.06±0.03[a]	1.03±0.13[a]	1.01±0.03[a]	0.84±0.11[a]
VFI	2.34±0.03[a]	2.44±0.08[a]	2.45±0.18[a]	2.48±0.08[a]	2.58±0.16[a]	2.55±0.01[a]	2.67±0.10[a]

Values are the mean±S.D. of duplicate groups. In the same line, values with different superscripts are significantly different ($P<0.05$).
IBW–initial body weight.
DGI–daily growth index: $100\times(\text{Final body weight}^{1/3}-\text{Initial body weight}^{1/3})/\text{time in days}$.
FCR–feed conversion ratio=dry feed intake/weight gain.
PER–protein efficiency ratio=wet weight gain/crude protein intake.
VFI–voluntary feed intake=100×dry feed intake/mean body weight/time in days.

difference (HSD). Significant differences were considered when $P<0.05$.

3. Results

3.1. Growth performance and feed utilization

Data on growth performance and feed utilization of sea bass fed the experimental diets are reported in Table 3. Final weight and growth rate were significantly reduced ($P<0.05$) only in fish fed the GC-10 diet. These fish also showed the lowest ($P<0.05$) daily weight gain (% initial body weight), whereas feed conversion ratio displayed significantly higher ($P<0.05$) values than those fed the control diet. The inclusion of seaweeds in the diets had no significant effect on PER or voluntary feed intake ($P>0.05$).

3.2. Digestibility

Apparent digestibility coefficients (ADC) for sea bass are presented in Table 4. The ADCs of dry matter and fat were significantly lower ($P<0.05$) only in fish fed the GC-10 diet. The inclusion of other seaweeds (*G. bursa-pastoris* and *U. rigida*), either at 5% or 10% inclusion level did not affect ADC values. On the other hand, the ADCs of protein were not affected by any of the seaweeds.

3.3. Body composition and nutrient retention

At the end of the 10-week trial, body composition of European sea bass (Table 5) did not show any significant variation between treatments, except for fish fed GC-10 that exhibited significantly higher ($P>0.05$) ash content than those fed the control diet. Dry matter, protein and lipid retention did not vary with seaweeds inclusion.

4. Discussion

The potential use of macro algae in fish feeds depends on costs involved in their production, harvesting and processing prior to their inclusion in fish diets. In this experiment, relatively low costs were incurred in seaweeds production, as they can be considered as by-products of this industry, once they were used as biofilters in effluents of fish farms before being incorporated in fish diets.

The present results evidenced that the increasing incorporation of both *G. bursa-pastoris* (GP) and *U. rigida* (UR), from 5% to 10%, did not affect growth performance or feed efficiency of European sea bass.

Table 4
Apparent digestibility coefficients (ADC) of the three experimental diets in European sea bass

	Diet						
	Control	GP-5	GP-10	UR-5	UR-10	GC-5	GC-10
Dry matter (%)	68.07±0.02[a,b]	67.03±0.30[a,b]	69.99±0.81[a]	69.05±1.80[a]	66.60±2.28[a,b]	67.29±0.13[a,b]	64.19±0.99[b]
Protein (%)	93.38±0.14[a]	93.50±0.02[a]	94.03±0.04[a]	92.42±0.34[a]	91.03±0.53[a]	90.70±1.68[a]	87.10±4.26[a]
Lipid (%)	99.27±0.63[a]	98.66±1.75[a]	99.77±0.11[a]	98.12±0.02[a]	98.83±0.62[a]	98.78±0.78[a]	92.81±1.73[b]

Values are the mean±S.D. of triplicate groups. In the same line, values with different superscripts are significantly different ($P<0.05$).

Table 5
Whole body composition and nutrient retention in European sea bass juveniles fed the three experimental diets for 10 weeks

	Diet						
	Control	GP-5	GP-10	UR-5	UR-10	GC-5	GC-10
*Final body composition**							
Dry matter (%)	30.71±1.05[a]	30.45±0.06[a]	30.49±0.73[a]	31.54±0.95[a]	31.45±0.07[a]	32.03±0.63[a]	29.57±1.56[a]
Protein (% DM)	51.15±0.18[a]	50.34±4.31[a]	54.38±0.58[a]	53.28±1.51[a]	53.39±0.40[a]	51.01±0.18[a]	53.55±3.87[a]
Lipid (% DM)	30.67±0.47[a]	27.00±8.96[a]	32.06±1.65[a]	32.39±2.28[a]	30.67±1.73[a]	33.56±1.41[a]	27.22±2.20[a]
Ash (% DM)	12.78±0.31[a]	13.72±0.55[a,b]	13.94±1.18[a,b]	13.61±0.39[a,b]	14.37±0.24[a,b]	13.67±0.31[a,b]	15.78±1.41[b]
Retention (% of intake)							
Dry matter	19.26±1.04[a]	18.44±1.60[a]	19.61±2.91[a]	17.94±1.38[a]	16.84±2.13[a]	17.81±0.09[a]	11.77±2.83[a]
Protein	15.34±0.80[a]	15.13±3.49[a]	18.89±2.55[a]	16.57±0.55[a]	16.08±2.30[a]	15.05±0.19[a]	11.40±1.24[a]
Lipid	46.64±3.26[a]	38.33±21.87[a]	53.57±11.09[a]	48.46±8.27[a]	42.87±8.49[a]	49.52±3.15[a]	25.69±9.68[a]

Values are the mean±S.D. of duplicate groups. In the same line, values with different superscripts are significantly different ($P<0.05$).
DM–dry matter.
Nutrient retention=nutrient gain/nutrient intake, %.
*Initial body composition (%) was: dry matter, 28.92; protein, 56.41; lipid, 26.10; ash, 17.56.

Daily weight gain and growth rate have even showed the highest value for fish fed GP-10. Previous works with snakehead (*Channa striatus*) fry showed that the incorporation of 5% *Ulva* spp. resulted in increased growth rate, feed efficiency and feed consumption, but the same level of *Gracilaria* spp. had no significant effect on those parameters (Hashim and Mat Saat, 1992). Moreover, Mustafa et al. (1995) found that the inclusion of three different seaweeds (*Ascophyllum nodosum*, *Porphyra yezeoensis* and *Ulva pertusa*) at a level of 5% increased body weight, feed utilization and muscle protein deposition in red sea bream fingerlings (*Pagrus major*). Nonetheless, Appler (1985) reported similar growth performances and protein utilization efficiencies in *Oreocromis niloticus* and *Tilapia zillii* when fed diets containing 5% of a freshwater algae *Hydrodictyon reticulatum*, but reduced growth performances with increasing dietary inclusion levels. Similarly, Davies et al. (1997) demonstrated that the use of the macroalgae *Porphyra purpurea* at high inclusion levels (16% and 33%), as an ingredient for mullet (*Chelon labrosus*) diets suppresses growth performance and feed utilization efficiency. Contradictory results were obtained by El-Sayed (1994), who evaluated the use of a microalga meal, *Spirulina*, as protein source for silver sea bream (*Rhabdosargus sarba*) fingerlings at inclusion levels up to 100% and concluded it could successfully replace up to 75% of the fish meal protein without any adverse effects on growth performance and feed utilization efficiency. These results suggested that the response of fish to algae seems to be species-specific and indicate a general beneficial effect of low level supplementation of seaweeds in fish diets. The relative low nutritive value of seaweeds could explain their deleterious effect on overall growth performance at high inclusion levels, and could also explain the lowest performance of fish fed the GC-10 diet. Wahbeh (1997) analysed the nutritional quality of several algae and detected some amino acids deficiencies (*Ulva lactuta*, for example, lacked cysteine) and high ratios of n-6 to n-3 PUFAs. The author suggested that a mixture of several algae species could provide fish with an adequate supply of all essential amino acids and fatty acids composition that would result in increased growth performances.

In this study, European sea bass showed similar voluntary feed intake, independently of the seaweed tested or its inclusion level. The inclusion of vegetable protein in diets for fish has been associated with reduced feed intake and decreased growth performance (Davies et al., 1997; Dias, 1999). However, Hashim and Mat Saat (1992) have observed and enhanced feeding activity and greater feed consumption of snakehead fed 5% *Ulva* spp. meal diet, compared to other seaweeds, indicating the possible presence of a food attractant for this fish. The seaweeds used in our study did not seem to affect the palatability of the diet for a carnivorous fish like sea bass.

The utilization of a diet depends on the degree of digestion. A preliminary digestibility study with *G. cornea* has showed very promising results, revealing high digestibility levels for both dry matter (>60%) and protein (>90%) (unpublished data). In the present study, the overall digestibility of the diets was generally high and not affected by the inclusion of GP and UR up to 10% and GC at 5%. Apparent digestibility coefficients (ADCs) for protein and fat did not vary significantly among those treatments and were in agreement to ADCs for carnivore species (NRC, 1993). Appler (1985) reported decreasing ADCs of protein with increasing inclusion levels of *H. reticulatum* in *O. niloticus* and *T. zillii*. Nonetheless, at 5% and 10% inclusion

levels, ADCs of protein were similar to those obtained in the present study (ADC about 90%). The lower digestibility obtained for fish fed GC-10 could be attributable in part to the possible presence of algal material in this species. The partial replacement of fish meal by GP and UR up to 10%, and by GC at 5% did not affect the whole-body composition of sea bass, whereas fish fed GC-10 exhibited higher ash content. Moreover, both protein utilization and retention ratios were reduced in fish fed GC-10 which was reflected in significantly lower growth rates.

Our findings clearly show that macro algae such as *G. bursa-pastoris*, *U. rigida* and *G. cornea* have great potential as alternative ingredients in diets for European sea bass juveniles at dietary inclusion levels up to 10% for *G. bursa-pastoris* and *U. rigida* and up to 5% inclusion level for *G. cornea* with no adverse effects on growth performance and feed utilization efficiency. A more comprehensive research in future works is, however, needed to evaluate the efficacy of such products in longer term feeding trials and to determine the optimum dietary inclusion levels of these seaweeds in fish diets, without compromising the growth performance and final product quality. It would also be interesting to access the seaweed cost per kg, in comparison to fish meal, to assess whether low inclusion levels could be cost effective at a commercial scale.

Acknowledgements

The authors appreciate the help of António Júlio Pinto and João Lima for their assistance during the growth trial and of Claúdia Silva for performing analytical work. The EU Project Q5RS-2000-31334 supported this study.

References

Alexis, M.N., 1997. Fish meal and fish oil replacers in Mediterranean marine fish diets. In: Tacon, A.G.J., Basurco, B. (Eds.), Feeding Tomorrow's FishCahiers Options Mèditerranéennes, Zaragoza: CIHEAM-IAMZ, vol. 22, pp. 183–204.

Appler, H.N., 1985. Evaluation of *Hydrodictyon reticulatum* as protein source in feeds for *Oreocromis* (Tilapia) *niloticus* and *Tilapia zillii*. J. Fish Biol. 27, 327–334.

Buschmann, A.H., Correa, J.A., Westermeier, R., Hernández-González, M., Norambuena, R., 2001. Red algal farming in Chile: a review. Aquaculture 194, 203–220.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25–41.

Da Silva, J.G., Oliva-Teles, A., 1998. Apparent digestibility coefficients of feedstuffs in seabass (*Dicentrarchus labrax*) juveniles. Aquat. Living Resour. 11, 187–191.

Davies, S.J., Brown, M.T., Camilleri, M., 1997. Preliminary assessment of the seaweed *Porphyra purpurea* in artificial diets for thick-lipped grey mullet (*Chelon labrosus*). Aquaculture 152, 249–258.

Dias, 1999. Lipid deposition in rainbow trout (*Oncorhynchus mykiss*) and European seabass (*Dicentrarchus labrax*): nutritional regulation of hepatic lipogenesis. PhD Thesis, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto.

El-Sayed, A.-F.M., 1994. Evaluation of soybean meal, *Spirulina* meal and chicken offal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings. Aquaculture 127, 169–176.

El-Sayed, A.-F.M., 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. Aquaculture 179, 149–168.

FAO, 2002. The State of the World Fisheries and Aquaculture. FAO, Rome, Italy.

Furukawa, A., Tsukahara, H., 1966. On the acid method for determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bull. Jpn. Sci. Fish. 32, 502–506.

Gouveia, A., Davies, S.J., 1998. Preliminary nutritional evaluation of pea seed meal (*Pisum sativum*) for juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture 166, 311–320.

Gouveia, A., Davies, S.J., 2000. Inclusion of an extruded dehulled pea seed meal in diets for juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture 182, 183–193.

Gouveia, L., Rema, P., Pereira, O., Empis, J., 2003. Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgal biomass. Aquacult. Nutr. 9, 123–129.

Hashim, R., Mat Saat, N.A., 1992. The utilization of seaweed meals as binding agents in pelleted feeds for snakehead (*Channa striatus*) fry and their effects on growth. Aquaculture 108, 299–308.

Kaushik, S.J., Covès, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquaculture 230, 391–404.

Lahaye, M., Gomez-Pinchetti, J.L., Rio, M.J., Garcia-Reina, G., 1995. Natural decoloration, composition and increase in dietary fibre content of an edible marine algae, *Ulva rigida* (Chlorophyta) grown under different nitrogen conditions. J. Sci. Food. Agric. 68, 99–104.

Lovell, R.T., 2002. Diet and fish husbandry. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press, San Diego, pp. 703–754.

Maynard, L.A., Loosli, J.K., 1969. Animal Nutrition. McGraw-Hill, New York, p. 613.

Mustafa, M.G., Wakamatsu, S., Takeda, T.A., Umino, T., Nakagawa, H., 1995. Effects of algae meal as feed additive on growth, feed efficiency, and body composition in red-sea bream. Fish. Sci. 6, 25–28.

Nagler, P.L., Glenn, E.P., Nelson, S.G., Napolean, S., 2003. Effects of fertilization treatment and stocking density on the growth and production of the economic seaweed *Gracilaria parvispora* (Rhodophyta) in cage culture at Molokai, Hawaii. Aquaculture 219, 379–391.

Nakagawa, H., Kasahara, S., Sugiyama, T., 1987. Effect of Ulva meal supplementation on lipid metabolism of black sea bream, *Acanthopagrus sclegeli* (Bleeker). Aquaculture 62, 109–121.

NRC, 1993. Nutrient Requirements of Fish. National Research Council, National Academy Press, Washington, DC, p. 114.

Pinchetti, J.L.G., Fernández, E.C., Díez, P.M., Reina, G.G., 1998. Nitrogen availability influences the biochemical composition and photosynthesis of tank-cultivated *Ulva rigida* (Chlorophyta). J. Appl. Phycol. 10, 383–389.

Rupérez, P., Saura-Calixto, F., 2001. Dietary fibre and physicochemical properties of edible Spanish seaweeds. Eur. Food Res. Technol. 212, 349–354.

Sommer, T.R., D'Sousa, F.M.L., Morrissy, N.M., 1992. Pigmentation of adult rainbow trout, *Onchorhynchus mykiss*, using the green alga *Haematococcus pluvialis*. Aquaculture 106, 63–74.

Wahbeh, M.I., 1997. Amino acid and fatty acid profiles of four species of macroalgae from Aqaba and their suitability for use in fish diets. Aquaculture 159, 101–109.

Zar, J.H., 1996. Biostatistical Analysis. Prentice-Hall International Inc., London.