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Pterocladia (Rhodophyta) and *Ulva* (Chlorophyta) as feed supplements for European seabass, *Dicentrarchus labrax* L., fry

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Abstract The aim of this study was to evaluate the suitability of two marine macroalgae, *Ulva lactuca* (Chlorophyta) and *Pterocladia capillacea* (Rhodophyta), meals as a supplement to enhance the nutritional value of formulated feeds for European seabass *Dicentrarchus labrax* fry. Seven isoproteic (50 % crude protein), isocaloric (500 Kcal/100 g gross energy) diets containing four levels (0 or control, 5, 10, and 15 %) of either *Ulva* meal (UM) or *Pterocladia* meal (PM) were tested. Each diet was fed to triplicate groups of *D. labrax* fry (initial body weight, 0.23±0.02 g for *Ulva*- and 0.14±0.01 g for *Pterocladia*-fed fish), to apparent visual satiety for 8 weeks. The results indicated that feeding seabass at 5 % UM or PM level (U_5 and P_5 diets) produced the best growth, feed utilization, nutrient retention, and survival rates among all the dietary groups. Feeding fish with a 5 % PM-added diet has also improved stress response after a 5-min air exposure test, prior to the termination of the feeding trial. These findings suggest that both *Pterocladia* and *Ulva* meals could be potentially used as an additional feed component (at 5 %) for enhancement of seabass *D. labrax* fry performance, nutrients composition, and stress resistance especially when subjected to transportation from hatchery to weaning ponds/tanks.

Keywords European seabass · *Dicentrarchus labrax* · *Ulva* · *Pterocladia* · Feed additive

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Introduction

The need for nutritional sources safer than the traditional animal products for aquafeeds has recently renewed interest in ingredients that are generally derived from plant origin, mainly terrestrial and sometimes aquatic. Extensive efforts has been paid worldwide, over the past decade, in evaluating a wide range of novel ingredients either as protein sources or as feed additives for use in fish feeds. Currently, various plant protein resources are major candidates in fish diets due to availability and low cost. Algae have been used for nutritional purposes in animal and human diets since a very early date (Montgomery and Gerking 1980) and recently increasing interest has arisen and is expected to grow in the years to come. Nowadays, algae (micro and macro) are utilized diversely as aquaculture feeds, but their main applications are related to nutrition. In the meantime, macroalgae have been credited as a source of dietary protein for fish (Valente et al. 2006; Dantagnan et al. 2009; Ragaza et al. 2012), amino acids, fatty acids (Wahbeh 1997; Soler-Vila et al. 2009), vitamins and minerals, coloring agent (Kissel et al. 1992; Soler-Vila et al. 2009), and other biologically active phytochemicals (Mustafa and Nakagawa 1995; Nakagawa 1997). The potential of macroalgae as an alternate protein or feed supplement ingredient in aquatic feeds is currently being examined in many parts of the world (Shields and Lupatsch 2012), because of their high protein content and production rate (Mustafa and Nakagawa 1995). Prior studies have reported that supplementing fish feed with algae meal had resulted in a considerable enhancement of growth rate, diet utilization, flesh quality, and disease resistance (Wassef et al. 2001, 2005; Valente et al. 2006; Dantagnan et al. 2009; Ergün et al. 2009; Güroy et al. 2011; Wan et al. 2012; Ragaza et al. 2012) and further indicated favorable use as a binder (Hashim and Mat Saat 1992) and

feed attractant for several fish species (Nakajima et al. 1990; Nakajima 1991; Shields and Lupatsch 2012).

Nutritional studies evaluating algae as a major feed ingredient for farmed fish are currently fewer in number. Nevertheless, the need for evaluation of common marine macroalgae, as a dietary ingredient, is crucial to nutritional research and feed development for farmed fish species. Therefore, further research for thorough evaluation is required in order to determine their nutritional value, appropriate use levels in prospective diets, and bioavailability of different nutrient classes to fish.

European seabass, *Dicentrarchus labrax* (a strictly carnivorous fish) is a highly valuable commercial species cultured in Europe, particularly in the Mediterranean region. Recently, and as a result of technological advances, seabass aquaculture has similarly expanded in Egypt. The landing of seabass from capture fisheries has been declining or even remained static during the past few years. On the other hand, the culture of European seabass exhibited a dramatic increase during these years, in order to meet the increasing consumers' and exportation demands. However, the challenge that faces seabass nutritionists is the development of commercial, cost-effective feeds using locally available, cheap, and unconventional feed ingredients. Recently, a great attention has been paid to the use of plant origin ingredients, including macroalgae, within seabass feeds (Kaushik et al. 2004; Tibaldi et al. 2006; Valente et al. 2006).

The green alga *Ulva lactuca* (Chlorophyta) and red alga *Pterocladia capillacea* (Rhodophyta) are among the dominant macroalgae along the Egyptian Mediterranean coast all the year around. They grow near the water level, in large amounts, and can easily be harvested by hand, from natural populations. Previous similar feeding trials with the same algae meal of *U. lactuca* with grey mullet (*Mugil cephalus*) fingerlings and of *U. lactuca* and *Pterocladia capillacea* with gilthead seabream (*Sparus aurata*) fry (Wassef et al. 2001, 2005) were successful. In addition, utilization of *Ulva rigida* by European seabass juveniles was evidenced to have no negative consequences on fish performance (Valente et al. 2006). Besides, more recent studies on *Ulva* meal supplementation in Nile tilapia (*Oreochromis niloticus*) (Ergün et al. 2009) and rainbow trout (*Oncorhynchus mykiss*) diets (Güroy et al. 2011) have been evidenced to be advantageous. Moreover, the suitability of the incorporation of the red alga *Pterocladia capillacea* in fish feeds has not been previously evaluated, except for gilthead seabream fry. In the meantime, the effects of including these algae meals (*U. lactuca* and *Pterocladia capillacea*) in European seabass feeds, particularly at earlier ages, are still unknown. Therefore, the present study was initiated to investigate the dietary inclusion of the green alga *Ulva lactuca* or red alga *Pterocladia capillacea* meal to enhance the nutritional value of formulated feeds of seabass, *D. labrax* fry. This study has also a significant practical implication in seabass nutrition, since the existing information on algal supplementation in their diets is still limited.

Materials and methods

The present experiment was carried out in Fish Nutrition Laboratory, National Institute of Oceanography and Fisheries, Alexandria, Egypt. From a local marine fish hatchery, 2,400 hatchery-bred European seabass, *D. labrax* fry (60 d old), were obtained. Fish were stocked in triplicates within 24 glass aquaria/tanks (120 L each), supplied with a natural seawater flow-through system, continuous aeration, and biological filters, at a density of 100 fry per tank. Fish were acclimated to laboratory conditions for 2 weeks, prior to the initiation of the feeding trial. During the conditioning period, the fish were fed with a 50 % crude protein (CP) commercial diet. At the end of the acclimation period, fish in each tank were counted and reweighed collectively and the mean initial body weight (BW_i) for each of the eight dietary groups was recorded: mean BW_i=0.14±0.01 g for *Pterocladia*-fed or BW_i=0.23±0.02 g for *Ulva*-fed fish groups. Temperature, salinity, dissolved oxygen, and pH were monitored and records were 20–22 °C, 34–35 ppt, 8.2–8.8 mg L^{-1}, and 7.8–8.1, respectively, throughout the experimental period with a 12:12-h light/dark cycle.

Experimental diets

Fresh *Ulva lactuca* (Chlorophyta) and *Pterocladia capillacea* (Rhodophyta) were collected from the Mediterranean coastal waters off Alexandria, Egypt. The algae were thoroughly washed with fresh water, sun-dried for 2–3 h, then oven-dried at 60 °C until complete dryness (for 48 h), ground into powder, and sieved, and their chemical composition was determined (Table 1). Amino acid and fatty acid profiles of both algae meals, of the same batch, have been reported earlier by Wassef et al. (2005), and their mineral content by Abdallah (2008). Seven isonitrogenous (50 % CP), isocaloric (500 Kcal/100 g gross energy), fish meal-based diets were prepared in the laboratory. Either *Ulva* meal (UM) or *Pterocladia* meal (PM) was included as a feed additive at four levels: 0 (control), 5, 10, and 15 % in diets designated as CTRL (U_0/P_0), U_5, U_{10}, and U_{15} or P_5, P_{10}, and P_{15} for UM or PM, respectively. Diets were stored at −4 °C until used. The composition and proximate analyses of experimental diets are shown in Table 2. The diets were fed to the eight dietary groups of seabass fry in triplicates for each diet, three to five times per day, to apparent visual satiety over a period of 8 weeks. The amount of feed consumed in each tank was daily calculated, and feed intake for each treatment was accordingly obtained.

Table 1 Composition (mean ± SD, %DM) of *U. lactuca* and *Pterocladia capillacea* meals

Composition	Ulva meal (UM)	Pterocladia meal (PM)
Crude protein (CP)	17.44±0.20	22.61±0.20
Lipids	2.50±0.10	2.18±0.10
Ash	32.85±0.30	37.30±0.20
Crude fiber	5.74±0.08	9.62±0.11
Nitrogen free extract (NFE)[a]	41.47±0.20	28.29±0.15
Moisture	3.69±0.05	3.05±0.10
Gross energy (GE, Kcal/100 g)	315	55
Protein/E[b] ratio	303	75
Phosphorus (P) (mg)[c]	414.3±26.1	265.0±6.6
Sodium (Na) (mg)[c]	925.0±16.1	855.0±21.1
Calcium (Ca) (mg)[c]	3216.0±69.6	7504.0±9.3
Iron (Fe) (mg)[c]	119.7±24.2	56.8±13.4

[a] Calculated by difference

[b] Expressed in mg $Kcal^{-1}$ GE

[c] Abdallah (2008)

Data collection and chemical analyses

At the end of the study, fish within each tank were weighed in bulk and average final body weight (BW_f, g) for each treatment was recorded. Survival rate was also calculated.

A pooled sample of 12 fish from the initial group and five fish per tank at the end of the trial were sacrificed and frozen (−40 °C) for subsequent body composition analyses. Moisture, protein, lipid, ash, and fiber contents were determined according to the AOAC (1995) standard methodology. Diet and fish samples (n=3) were analyzed for dry matter (DM, 24 h at 105 °C), crude protein (N × 6.25, using the Kjeldahl method), lipids (a mixture of chloroform and methanol, 2:1 *v*/*v* in a Soxhlet apparatus), and ash (at 600 °C for 15 h) contents. Gross energy content was calculated based on 5.59, 9.37, and 4.11 Kcal g^{-1} for protein, lipid, and carbohydrates, respectively.

Growth rate and feed utilization efficiency indices were calculated as follows: total weight gain, $WG=BW_f-BW_i$ (g); percent weight gain, $PWG=100\ (BW_f-BW_i)/BW_i$ (%); specific growth rate, $SGR\ (\%/d)=100\ (\ln BW_f-\ln BW_i)/t$, where BW_f and BW_i are the final and initial fish weight (g), respectively, and t is the time of experiment in days; feed conversion ratio, FCR=dry feed intake/total weight gain (g/fish), protein efficiency ratio=weight gain (g)/protein intake (g); and protein productive value, PPV=100 (protein gain (g)/protein fed (g)).

Air exposure test

At the end of the feeding trial, a group of 10 fish from each tank were exposed to atmospheric air (as an induced stressor) for 5 min, then returned to oxygen-saturated fresh seawater, within each respective tank, and their recovery state was determined. According to Nakagawa et al. (1997), recovery is the state at which the fish swap up from their lateral side; therefore, the time from the succumbed condition was recorded and percentage survival was accordingly calculated. This test was repeated three times for each dietary treatment (n=30), and mean survival rates were then recorded.

Statistical analysis

Data are presented as means ± standard deviation (SD). One-way ANOVA was used to test the effects of algal inclusion on fish performance, body composition, and stress resistance indices. Duncan's multiple range test

Table 2 Ingredients and proximate analysis (%DM) of experimental diets

Ingredient	CTRL	U_5	U_{10}	U_{15}	P_5	P_{10}	P_{15}
Fish meal (FM)[a]	65	65	64	62	65	64	62
Ulva meal (UM)	–	5	10	15	–	–	–
Pterocladia meal (PM)	–	–	–	–	5	10	15
Wheat flour (WF)	30	25	21	18	25	21	18
Fish oil (FO)[b]	3	3	3	3	3	3	3
Vit. and min. premix[c]	2	2	2	2	2	2	2
Crude protein (CP)	50.20	50.86	50.55	50.12	50.50	50.23	49.85
Lipids	12.82	12.54	12.25	11.15	12.29	11.61	11.49
Ash	9.85	12.29	13.57	14.67	10.48	11.19	12.56
Fiber	0.67	1.08	1.31	1.88	1.15	1.52	1.74
Nitrogen-free extract (NFE)[d]	26.46	23.23	22.32	22.18	25.58	25.45	24.36
Gross energy (Kcal/100 g)	512	502	494	484	507	500	494
P/E ratio (mg $Kcal^{-1}$ GE)	98	101	102	104	100	100	101

[a] Danish 999

[b] Iceland

[c] NRC (1993)

[d] Calculated by difference

was used to detect significant differences among treatments ($P<0.05$). All statistical analyses were conducted by using Statistics for Windows Software Package (version 4.5, 1995).

Results

PM has relatively higher protein, ash, and fiber contents than UM, whereas both meals contain almost equal amounts of lipids (Table 1). However, UM has a comparatively higher level of carbohydrates (nitrogen-free extract (NFE)) and energy than PM. Results of amino acid (AA) and fatty acid (FA) composition of both algae meals of the same batch (Wassef et al. 2005) showed also a similarity of total essential AA (43.5 or 43.2 g/100 g protein for PM or UM, respectively) and nonessential AA (49.4 % for PM and 47.8 % for UM) contents. Nineteen AAs were quantified, among which 10 AAs are essential in both algae meals.

Moreover, PM contains relatively higher total mono- (MUFA) and polyunsaturated FAs (MUFA=57.2 % and PUFA=11.3 % of total FAs) but lower total saturated FA (SFA=23.7 %) than those in UM (MUFA=32.3, PUFA=5.1, and SFA=62.5 %). PM contains higher levels of oleic (18:1 *n*−9, OLA=54.7 %) and linoleic (18:2 *n*−6, LOA=9.7 %) acids but lower levels of α-linolenic acid (18:3 *n*−3, LNA=0.1 %) than those in UM (OLA=31.1 %, LOA 1.0 %, LNA=4.1 %). Total *n*−6 PUFA is comparatively higher in PM (10.2 %) than in UM (1.0 %), whereas total *n*−3 PUFA is higher for UM (4.1 %) than for PM (1.0 %). Unexpectedly, only PM contains small quantities of eicosapentaenoic acid (20:5 *n*−3, EPA=0.3 %), docosahexaenoic acid (22:6 *n*−3, DHA=0.6 %), and arachidonic acid (20:4 *n*−6, ARA=0.46 %).

In addition, both PM and UM are rich in phosphorus, sodium, potassium, calcium, and magnesium (Table 1).

Growth performance and feed utilization

The results showed that voluntary feed intake (FI) was not significantly ($P>0.05$) affected by either algae meal addition or inclusion level, despite the slight increasing trend of FI values for UM-fed fish, compared to CTRL fish. Diet P_5 recorded the highest FI value, but nonsignificant, among all the treatments, which indicates slightly improved palatability for both P_5 and UM diets than the CTRL (alga-free) diet. Moreover, seabass fry fed with the U_5 diet exhibited a significantly ($P<0.05$) higher percentage weight gain (WG, g/fish), survival rate (SR, 85.2 %), PPV, and best FCR than those fed with the CTRL or other UM diets (Table 3). However, the inclusion of UM in the diets had no significant ($P>0.05$) effect on SGR (%/d) or protein efficiency ratio (PER) of all UM treatments. Likewise, the diet containing 5 % PM (P_5) recorded significantly ($P<0.05$) improved growth and feed utilization indices: BW_f, PWG, PER, and PPV as compared to the $CTRL_P$ or other PM-added diets. On the other hand, the highest survival rate (84.5 %) was obtained with fish fed with the P_{10} diet. Moreover, FCR and PPV were not significantly different ($P>0.05$) between P_5-and P_{10}-fed fish or $CTRL_P$ fish. Further addition of UM or PM to 15 % had resulted in a significant ($P<0.05$) deterioration in all growth and feed utilization indices relative to those of CTRL fish (Table 3). Therefore, feeding seabass fry with both P_5 and U_5 diets has significantly improved their growth, feed utilization, and survival rate.

Body composition and nutrient retention

The major nutrient composition of seabass fry fed with UM- or PM-added diet is summarized in Table 4. Generally, protein and ash contents were not significantly ($P>0.05$) different among treatments, except for the U_{15}-fed fish, which had the lowest ($P<0.05$) protein

Table 3 Growth and feed utilization indices (mean ± SD) of *D. labrax* fry fed with the experimental diets for 8 weeks

Index	U_0/CTRL	U_5	U_{10}	U_{15}	P_0/CTRL	P_5	P_{10}	P_{15}
BW_i (g)	0.22±0.03	0.24±0.04	0.21±0.05	0.25±0.20	0.14±0.02	0.16±0.02	0.12±0.00	0.15±0.00
BW_f (g)	0.55±0.10b	0.78±0.09a	0.66±0.10ab	0.62±0.02ab	0.94±0.20b	1.13±0.30a	0.64±0.00c	0.62±0.00c
PWG (%)	150±3.50c	225±5.00a	214±4.60b	148±2.50c	571±4.70b	606±4.80a	433±3.00c	313±3.20d
SGR (% d^{-1})	1.64±0.40	2.10±0.40	2.04±0.30	1.62±0.40	3.40±0.30	3.49±0.30	2.99±0.40	2.53±0.30
FI (g/fish)	0.39±0.02	0.53±0.01	0.51±0.01	0.71±0.02	0.84±0.12	0.94±0.25	0.83±0.15	0.77±0.16
FCR	1.18±0.90a	0.98±0.90a	1.13±0.90a	1.92±1.20b	1.05±0.20a	0.97±0.30a	1.21±0.20a	1.64±0.20b
PER	1.65±0.02	1.86±0.01	1.67±0.01	1.03±0.02	1.90±0.01a	2.02±0.02a	1.63±0.02b	1.21±0.01b
PPV	25.0±0.60b	31.06±0.50a	22.22±0.60b	13.89±0.50c	30.95±0.80a	35.42±0.80a	28.1±0.70a	20.51±0.60b
SR (%)	74.1±2.1b	85.2±1.9a	72.8±1.9b	65.8±2.2c	72.5±1.4c	76.6±1.9a	84.5±1.7a	78.5±2.0b

Means with the different letters in the same row for either *Ulva* (U) or *Pterocladia* (P) diet are significantly different ($P<0.05$)

BW_i initial body weight, BW_f final body weight, *PWG* % weight gain, *SGR* specific growth rate, *FI* feed intake, *FCR* feed conversion ratio, *PER* protein efficiency ratio, *PPV* protein productive value, *SR* survival rate

Table 4 Body composition (% wet weight) (mean ± SD) of *D. labrax* fry fed with the experimental diets for 8 weeks

Parameter	Initial	Final CTRL/U0	U5	U10	U15	CTRL/P0	P5	P10	P15
Protein	13.16±0.09	14.49±0.11a	15.27±0.10a	14.27±0.09a	12.93±0.08b	16.30±0.08	16.48±0.10	16.54±0.10	15.78±0.20
Lipids	7.04±0.05	6.04±0.06	6.43±0.10	6.59±0.05	6.27±0.10	4.21±0.06a	6.55±0.04b	6.24±0.20b	5.39±0.11ab
Ash	4.40±0.06	2.37±0.06	1.20±0.10	1.24±0.06	1.16±0.10	3.20±0.12	3.54±0.10	2.18±0.09	3.25±0.08
Moisture	75.60±0.2	78.45±0.30a	76.10±0.10b	77.03±0.09ab	79.92±0.20a	74.90±0.20a	72.95±0.10b	75.30±0.09a	75.65±0.20a

Values with different letters in the same row for *Ulva* (UM)- or *Pterocladia* (PM)-added diets are significantly different ($P<0.05$)

percentage among all the dietary groups. Fish fed with the PM diets recorded relatively higher protein and ash but lower moisture content than the those of the respective UM diet, whereas lipid content was almost equal in all the dietary groups. The inclusion of dietary UM did not significantly ($P>0.05$) affect the lipid or ash contents of fish; however, a decrease ($P<0.05$) in moisture content was noticed at 5 % level in comparison with the CTRL fish. Although dietary addition of PM did not significantly altered protein or ash contents of fish, it has led to a significant ($P<0.05$) increase in lipid content at 5 and 10 % levels and a decrease in moisture content of only P_5-fed fish compared to CTRL fish.

Air exposure (stress resistance) test

SRs of seabass fry exposed to atmospheric air test for 5 min (as an induced stressor), at the end of the feeding trial, are presented in Table 5. Generally, dietary PM supplementation had led to improvement of fish survival compared to the CTRL alga-free diet, whereas UM addition had no effect. Higher ($P<0.05$) survival rates (66 and 65 %) were obtained for fish fed with the P_{10} and P_5 diets, in comparison with those fed with the CTRL diet (55 %); thereafter, a significant ($P<0.05$) reduction in SR was noticed for P_{15}-fed fish. On the contrary, the U_5-fed fish recorded a survival rate (20 %) comparable to that of CTRL; then SR was further decreased ($P<0.05$) for U_{10}- and U_{15}-fed fish (10 %), indicating a negative effect for UM addition on fish stress resistance.

Table 5 Survival rates (%) of *D. labrax* exposed to atmospheric air for 5 min, after feeding on UM- or PM-added diet for 8 weeks

Algae meal	Dietary level (%)			
	0 (CTRL)	5	10	15
UM	20±1.41a	20±0.09a	10±1.1b	10±0.15b
PM	55±1.71a	65±2.12b	66±2.26b	55±1.92a

Values with the same letter in the same row for either *Ulva* (UM)- or *Pterocladia* (PM)-supplemented diets are not significantly different ($P<0.05$)

Discussion

Nutritional studies evaluating macroalgae, as a feed ingredient for farmed fish, are relatively limited; however, several scientific research studies have highlighted their great potential particularly for omnivorous and herbivorous species. A number of green, red, and brown algae meals can provide an important, direct or indirect, feed source for many farmed fish species. The usefulness of macroalgae as a dietary ingredient for marine fish feeds has been investigated. Macroalgae have been credited to have considerable positive effects on fish performance, immune system, lipid metabolism (Mustafa and Nakagawa 1995; Güroy et al. 2011), and stress resistance (Nakagawa et al. 1997; Wassef et al. 2005) besides providing a source of protein, amino acids, fatty acids, vitamins and minerals, and other biologically active phytochemicals (reviewed by Shields and Lupatsch 2012). Several studies have described the effects of inclusion of different seaweeds at various levels, within compounded aquafeeds. Prior studies have shown that a low level incorporation of algae meal (3–5 %) in compounded feeds promoted growth, diet utilization, immune response, and flesh quality in several fish species (Mustafa and Nakagawa 1995; Nakagawa et al. 1997; Wassef et al. 2005; Ergün et al. 2009; Dantagnan et al. 2009; Güroy et al. 2011; Ragaza et al. 2012; and others). Other reports have similarly indicated the same beneficial effects with the inclusion level of 5–10 % at most of marine macroalgae meal in feed for European seabass juveniles (Valente et al. 2006) and 6 % or up to 10 % for rainbow trout, *Oncorhynchus mykiss* of different sizes (Dantagnan et al. 2009; Soler-Vila et al. 2009). Alongside this line, the present study suggested the inclusion of 5 % *U. lactuca* or *P. capillacea* meal as a dietary supplement for seabass, *D. labrax*, fry for boosting fish performance and stress resistance. These findings confirm the positive results reported on improved growth performance of gilthead seabream fry (*S. aurata*) with the addition of 5 % UM or 10 % PM to their diets (Wassef et al. 2005). On the contrary, Kissil et al. (1992) observed no significant effect after the inclusion of *Ulva* meal in the diets of grow-out gilthead seabream on growth rate but found a slightly better protein utilization at the highest tested level of 8 %. Our results are also in agreement with those reported

for Nile tilapia (*Oreochromis niloticus*) using 5 % *U. rigida* dietary supplementation for better growth, feed efficiency, and nutrient utilization (Ergün et al. 2009). In the present study, improvement of seabass fry performance and survival rate with the inclusion of 5 % UM or PM has been evidenced, probably due to the relatively high protein and mineral contents (Table 1) and good essential amino acid profile of both algae meals (Wassef et al. 2005). Earlier data on proximate nutrients and fatty acid profile of *U. lactuca* from different batches in other locations have further confirmed our results (Luistro et al. 1987; Wong and Cheung 2000). On the other hand, the use of macroalgae as the sole dietary protein component had resulted in malformation and impaired growth in carps (Meske and Pfeffer 1978).

The inclusion of plant-derived proteins in carnivore fish diets has been reported to reduce daily feed intake. The poor palatability with consequent reduction of voluntary feed intake has been considered to be a reason for the poor performance of mullet (*Chelon labrosus*) fed with high levels of red alga, *Porphyra purpurea*, diets (Davies et al. 1997). In the mean time, other studies have reported the presence of dimethyl-β-propiothetin in *Ulva* spp., a chemical substance that operates as a feed attractant for fish (Nakajima et al. 1990; Nakajima 1991, 1992). In contrast, the present study showed no significant effect in voluntary feed consumption among treatments, indicating no effect on feed palatability regardless of the algae meal tested or its incorporation level. Similar results were also obtained for European seabass juveniles fed with three different algae meals at two inclusion levels (5 and 10 %) (Valente et al. 2006).

In the present study, seabass fry fed with the highest algae meal addition exhibited inferior growth (P_{10} and P_{15} diets), retarded feed efficiency (U_{15} and P_{15} diets), or sometimes lower protein content (only U_{15} diet) in comparison with the CTRL fish. These notions are presumably due to (1) the presence of relatively high indigestible components such as polysaccharide fibers (%fibers=1.52, 1.74, and 1.88 % in P_{10}, P_{15}, and U_{15} diets, respectively, compared to 0.67 % in the CTRL diet) which might have suppressed nutrient absorption. Yone et al. (1986a) reported that excessive amount of dietary seaweed supplementation may have reduced the absorption of nutrients and resulted in inferior growth rate and feed utilization efficiency in red seabream (*Pagrus major*). Unfortunately, information on digestibility of major nutrients from marine macroalgae, for European seabass, during various life stages, is scarce. The apparent digestibility coefficient (ADC) of dry matter and lipid was significantly lower in European seabass juveniles fed with 10 % *Gracilaria cornea* relative to those fed with the CTRL (alga-free) diet. A preliminary digestibility study with *G. cornea* has shown digestibility levels >60 % for dry matter and >90 % for protein (Valente et al. 2006). In rainbow trout, ADC of dry matter decreased with the increase of red alga, *Porphyra dioica*, meal inclusion level to 15 % (Soler-Vila et al. 2009). Another study with two Tilapias, *Oreochromis niloticus* and *Tilapia zillii*, reported a decreasing ADC of protein with increasing inclusion levels of alga *Hydrodictyon reticulatum* (Appler 1985) and (2) high dietary carbohydrate content (NFE ranged from 22.2 to 25.5 % in P_{10}, P_{15}, and U_{15} diets), while carnivore seabass have a limited ability to utilize them particularly at small size, due to low amylolytic enzyme activity (Dias et al. 1998). From the nutritional point of view, the dehydrated macroalgae meal is an ingredient low in calories, with a high concentration of minerals and rich in carbohydrates, which are low digestible (Castro et al. 1994). Besides, soluble carbohydrate content in seaweeds has been estimated to range between 3.5 and 33.8 % (Kaehler and Kennish 1996). These notions are also presumably due to (3) the presence of anti-nutrients that have been described in seaweeds such as lectins and tannins (polyphenolic functional group) which interfere with feed utilization and digestion and may affect growth of fish (Francis et al. 2001). However, no data are currently available to support or deny this latter assumption. In view of scarcity on ADC data of major nutrient classes in macroalgae that have potential usage in aquafeeds, research in this context would be of considerable interest. Our results are in agreement with other studies which reported fish performance depression with high dietary inclusion levels: for European seabass juveniles fed with 10 % *G. cornea* (Valente et al. 2006), for rainbow trout fed with 15 % *Porphyra dioica* (Soler-Vila et al. 2009), and for mullets (*C. labrosus*) fed with 16 and 33 % *Porphyra purpurea* (Davies et al. 1997).

Although the use of macroalgae as an additional component in fish feeds has been investigated, information on how it could influence body or muscle composition of fish is relatively few. The present study has also shown no major alteration in body nutrient composition of seabass fry fed with algae meal, except for fish fed with the U_{15} diet which recorded the lowest protein content among all the dietary groups. PM addition at 5 and 10 % has increased the lipid content in the fish body, whereas moisture quantity was decreased at 5 % of either PM or UM, an advantage in relation to the firmness of fish muscles. These results are in accordance with those reported for rainbow trout, which showed a significant increment in lipids and reduction in protein contents of muscles with the highest (6 %) macroalgae, *Macrocystis pyrifera*, addition (Dantagnan et al. 2009). They also emphasized that the use of algae meal (at 3–6 %) might help to increase lipid quality of fish due to the beneficial effects of PUFAs for human health. Our results are also in partial agreement with those obtained for European seabass juveniles which revealed no major

changes in proximate nutrients with seaweed inclusion level or type, except for the higher ash content in fish fed with 10 % *G. cornea* (Valente et al. 2006). Similar earlier results for red seabream (*Pagrus major*) and black seabream (*Acanthopagrus schlegeli*) showed that *Ulva pertusa* meal supplementation contributed to the lipid deposition in muscle due to activation of lipid metabolism (Yone et al. 1986; Nakagawa et al. 1997). Meanwhile, it has also been suggested that dietary algae accelerate the assimilation of ascorbic acid in fish and improve the physiological conditions related to vitamin C metabolism, leading to the improvement of lipid metabolism (Nakagawa 1997; Dantagnan et al. 2009). More recently, Sáez et al. (2012) found that *U. rigida* and *G. cornea* did not produce qualitative changes in the composition of alkaline proteases in gilthead seabream and concluded that they have no effect on gut function. However, it appears that the effect of dietary seaweeds on body composition and lipid metabolism is a species-specific criterion, and the exact mechanisms involved in the efficacy of macroalgae as a fish feed additive are not yet clear.

These various results suggested that the response of fish to dietary algae seems to be species-specific as the major biochemical constituents of tested algae are different and their effect varied depending on type of algae and fish size.

The results of the present study have further indicated that incorporation of 5–10 % dietary PM had promoted stress resistance of fish compared to the CTRL ones (without algae meal), when a stressor like exposure to atmospheric air was induced at the end of the feeding trial. UM addition had no influence on fish stress resistance. This notion may be linked to the variation in ARA (20:4 $n-6$) content of different tested diets, since PM contained ARA (0.46 % of total fatty acids), while UM is devoid of this FA. Accordingly, all UM-added diets had almost similar ARA content (6.7 %), which was lower than those of the PM-added diets (data are not presented herein). In this context, the highest survival rate (best stress resistance) of seabass fry fed with the P_5 or P_{10} diet among all the treatments may be referred to their relatively high ARA content (7.51 and 7.45 % of total FAs) in comparison to the P_{15} diet (6.77 %). This fatty acid, which plays an important role in the regulation of stress response, is a precursor of eicosanoids generally produced in response to stressful situations. Koven et al. (2001) suggested that dietary ARA fed to gilthead seabream prior to handling process improved fish survival more effectively than when fed following handling stress. These findings on PM of the present study are in agreement with those reported on young red seabream (*Pagrus major*) which presented higher stress tolerance and faster recovery from anesthesia when fed with the alga *Ascophyllum* diets than those fed with the alga-free diet (Nakagawa et al. 1997).

On the basis of the present study results, it could be concluded that both green and red algae meals were found to be ingested and utilized by seabass fry; however, the red alga *Pterocladia capillacea* has better growth, feed utilization, survival, and stress resistance than the green alga *Ulva lactuca*. Both PM and UM could be potentially used as a feed additive at 5 % for seabass fry due to their local biomass availability and low cost; however, the mechanisms involved in the efficacy of these algae are not yet clear and further research is greatly needed in this regard.

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