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The red alga *Porphyra dioica* as a fish-feed ingredient for rainbow trout (*Oncorhynchus mykiss*): effects on growth, feed efficiency, and carcass composition

Anna Soler-Vila · Susan Coughlan · Michael D. Guiry · Stefan Kraan

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Abstract *Porphyra dioica* meal was added at levels of 5, 10 and 15% to a diet for rainbow trout formulated to be isonitrogenous and isolipidic. The control diet was a commercial trout diet without seaweed meal. The experimental groups were fed in triplicate for 12.5 weeks, during which fish weight increased on average from 107–261 g. Seaweed meal inclusion did not affect significantly weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER) and apparent digestibility coefficient of the dry matter (ADC_{dm}) for any of the diets. Voluntary feed intake (VFI) increased for all seaweed diets compared to the control diet but not significantly ($P>0.05$). Final weight (FW) was significantly smaller for the 15% *P. dioica* inclusion and hepatosomatic index (HSI) for the 10% and 15% inclusion. Carcass protein content increased for all three experimental diets, and was significantly higher for the diet with 10% seaweed inclusion. Rainbow trout fed with *Porphyra* meal presented a dark orange pigmentation of the flesh at the end of the trial, compared to the whitish color from the control fish. These results suggest that *P. dioica* can effectively be included in diets for rainbow trout up to 10% without significant negative effects on weight gain and growth performance. The pigmentation effect of the fish flesh by adding *P. dioica* meal to the feed is of a considerable interest to the organic salmon-farming industry.

Keywords Rainbow trout · Feed ingredient · Seaweed · *Porphyra dioica* · Pigment

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A. Soler-Vila (✉) · M. D. Guiry · S. Kraan
Irish Seaweed Centre, Martin Ryan Institute,
National University of Ireland,
Galway, Ireland
e-mail: anna.soler@nuigalway.ie

S. Coughlan
Carna Laboratories, MRI Carna,
Co. Galway, Ireland

Introduction

Fish farming is the fastest-growing sector of world food production. Fish meal and fish oil are key components of farmed fish diets. Aquaculture feed, especially feed for carnivorous species, is strongly dependent on fish meal and fish oil to meet critical protein and lipid requirements. Fish meal, representing the protein source, is the main ingredient in fish feed. Up to 1990, about 10% of the global fish meal production was used for aquaculture; however, this has tripled over the last decade and predictions for 2010 show values close to 45% (Hardy and Tacon 2002). Increasing use of fish for human consumption, along with a decline in availability and increasing costs of fish meal has created a need for alternative resources for the fish-farming industry as the current practice is not sustainable. If present trends are to continue, an urgent need has arisen to find alternative and sustainable feed ingredients (Hardy and Tacon 2002). Currently, various plant protein sources such as soybean, corn gluten, cotton seed and canola, are major candidates for the substitution of fish meal in fish diets due to availability and low cost. Generally, it has been observed that a partial substitution of fish meal with plant protein sources is well received by fish (Webster et al. 1997; Refstie et al. 1997). A level of inclusion of 48% of total protein as maize gluten in high-protein diets for small

salmon did not effect growth rates (Mente et al. 2003). Mundheim et al. (2004) found that up to 50% of total protein in fish feed for salmon can be of vegetable origin, and Morris et al. (2005) showed that in diets for rainbow trout, full-fat soybean meal could be included, providing 21% of the dietary crude protein without significant adverse effects on growth rate and survival.

Marine macroalgae have been harvested for many centuries for their nutritional and mineral content as part of human and animal food or for the functional properties of their polysaccharides (Fleurence 1999). Furthermore, a large number of studies have demonstrated the benefits and potential of macroalgae such as: an anti-obesity effect (Maeda et al. 2005); inhibition of tumor growth (Hirayasu et al. 2005); a source for biologically active compounds applied for prophylaxis and therapy of bacterial fish diseases (Bansemir et al. 2006); and, antioxidant activity (Yuan and Walsh 2006), although western cultures have been slow to realise their benefits. To date, little attention has been paid to the nutritional value of algae as a potential substitute of protein and other ingredients in fish feed such as: alginates (Sørensen and Denstadli 2008), as a source of pigments (Sommer et al. 1992), fatty acids (Atalah et al. 2007) and for their potential as feeding stimulants (Dworjanyn et al. 2007). Seaweeds are a valuable food source and contain significant quantities of proteins, lipids, vitamins and minerals (Sánchez-Machado et al. 2004; Marsham et al. 2007). The nutrient composition of seaweeds varies within species and is affected by geographical location and seasonality (Haggen Rødde et al. 2004). Most of the environmental parameters vary according to season and seaweeds vary their nutritional content in relation to several environmental factors such as water temperature, salinity, light and nutrients (Floreto et al. 1993; Marinho-Soriano et al. 2006).

The red algal genus *Porphyra* C. Agardh (Rhodophyta, Bangiales) also known as nori or laver, has high protein levels (Marsham et al. 2007) and is comparable with high-protein plant foods such as soybean, of which 70% has been reported to be digestible by humans (Arasaki and Arasaki 1983). Algae of this genus are also rich in iron, zinc, sodium, potassium and calcium (Sánchez-Machado et al. 2004; Dawczynski et al. 2007; Subba Rao et al. 2007). Previous work on inclusion of the seaweeds *Porphyra yezoensis* Ueda, *Ascophyllum nodosum* (Linnaeus) Le Jolis and *Ulva pertusa* Kjellman in the diets of fingerlings of red sea bream, *Pagrus major* (Temminck and Schlegel), showed an increase in body weight, feed utilisation and muscle protein deposition (Mustafa et al. 1995). Valente et al. (2006) showed that the green alga *Ulva rigida* C. Agardh and the red alga *Gracilaria bursa-pastoris* (S.G. Gmelin) P.C. Silva could be included at a 10% level in fish feed for European sea bass, *Dicentrarchus labrax* (Linnaeus) without negative consequences on growth performance, nutrient utilisation and body composition. On the other hand, *Gracilaria cornea* J. Agardh could only be included at a 5% inclusion rate. The suitability of the inclusion of *Porphyra dioica*, in fish feed for rainbow trout, *Oncorhynchus mykiss* (Walbaum) has not previously been reported. Thus, the main aim of the present study is: to evaluate the partial replacement of fish feed for rainbow trout with different percentages of *P. dioica* on growth performance, feed efficiency and carcass composition.

Materials and methods

Experimental site and trial conditions

A twelve-and-a-half week feed trial was conducted at Carna Laboratories, National University of Ireland, Co. Galway, from February to April 2005. Four different experimental diets were tested in 12×1,000 L tanks. The photoperiod was 12:12 h (light:dark) and water temperatures varied from 6 to 10°C over the course of the trial; dissolved oxygen and salinity were continuously monitored. Six hundred rainbow trout were obtained from a local fish farm. A 2-week acclimatisation period was undertaken followed by transfer from fresh to seawater. During the adaptation period, fish were fed a commercial diet. Rainbow trout (n=50) with an individual average weight of 108 g were randomly distributed over 12 tanks and each tank of fish was randomly assigned to a dietary group. Fish were individually weighed and, in order to minimise waste, fed by hand twice a day, according to body weight and operating temperature. Batch weights were taken every 2 weeks to allow adjustments of the feeding ratios. At the end of the feeding trial, fish were anaesthetised with a dose of MS222 (approx. 100 ppm). Faeces were stripped from ten fish per tank, while five fish per tank were individually measured and gutted and kept at −20°C for carcass analysis.

Biological and feed efficacy measurements

At the end of the experiment fish (n=600) were individually measured for: total length (TL), fork length (FL), total weight (TW), gutted weight (GW) and liver weight (LW). The following parameters were calculated for all diets:

HSI hepatosomatic index, $(\%\ \mathrm{g.g^{-1}}) =$ liver weight/body weight × 100.

DI Dressing Index, $(\%\ \mathrm{g.g^{-1}}) =$ eviscerated fish weight/body weight × 100

SGR specific growth rate, $\left(\%\ \text{body weight day}^{-1}\right) = (\ln w_2 - \ln w_1) \times 100/T$

(w_1 and w_2 are start and final weights respectively, ln is the natural logarithm and T the feeding days).

FE Feed Efficiency, ($g.g^{-1}$) = weight gain/feed intake.
FCR feed conversion ratio, ($g.g^{-1}$) = total dry feed intake / total weight gain.
PER protein efficiency ratio, ($g.g^{-1}$) = weight gain / crude protein intake.
CF condition factor, $(g.cm^{-1}) = \text{weight gain}/\text{fish length}^3 \times 100$.
VFI voluntary feed intake, $(g.kg^{-1}\ \text{mean BW/day}) = (Di \times 100/[(w_1 + w_2)/2]/T)$

(Di is dry matter intake; w_1 and w_2 are start and final weights respectively and T the feeding days)

PRE Protein retention efficiency (%) = 100 × (protein gain/protein intake)

where,

$$\text{Protein gain}\left(g.\text{mean BW}^{-1}\ \text{day}^{-1}\right) = 6.25 \times (w_2 \times N_2 - w_1 \times N_1)/T$$

$$\text{Protein intake}\left(g.\text{mean BW}^{-1}\ \text{day}^{-1}\right) = 6.25 \times (\text{VFI} \times \text{Nd})/T$$

(Nd is nitrogen content of the experimental diets)

Table 1 Ingredients and chemical composition of the experimental diets

Ingredients (%)	Control	P5	P10	P15
Fish meal	60.0	58.25	56.50	55.0
Fish oil	20.0	19.9	19.9	19.8
Porphyra dioica	0	5.0	10.0	15.0
Wheat starch	19.7	16.5	13.3	9.9
Vitamin premix	0.1	0.1	0.1	0.1
Mineral premix	0.1	0.1	0.1	0.1
Yttrium premix	0.1	0.1	0.1	0.1
Chemical composition				
Moisture (%)	6.1	5.6	5	4.6
Crude protein (%dm)	44.4	43.9	45.2	44.6
Lipid (%dm)	26.3	27.6	26.2	28.4
Carbohydrate (%dm)	14	19.9	18.1	14.7
Ash (%dm)	7.6	8.2	9	9.7

Vitamin premix (IU or $mg.kg^{-1}$ diet): Vit. A, 1998 IU; Vit. D3, 1500 IU; Vit. E, 150 IU; Vit. K3, 5 mg; Vit. B1, 8 mg; Vit. B2, 16 mg; pantothenic acid, 48 mg; niacin, 60 mg; biotin, 140 mcg; Vit. B12,12 mcg; folic acid, 3 mg; Vit, B6 16 mg; Vit C, 50 mg. Mineral premix (mg or $g.kg^{-1}$ diet): ferrous sulphate, 20 mg; copper sulphate, 3 mg; zinc sulphate, 100 mg; manganese sulphate, 15 mg; calcium-iodate, 2 mg; calcium total 0.2 g; phosphorus total, 0.001 g; potassium total 0.001 g.

Experimental diets

Four isonitrogenous and isolipidic experimental diets were manufactured by Skretting Aquaculture Research Centre, Stavanger, Norway. A total of 60 kg (d.w.) of *P. dioica* was collected from Spiddal, Co. Galway and dried and milled. *Porphyra* was included in the experimental diets at levels of 5, 10 and 15% and are indicated with P5, P10 and P15, respectively (Table 1). The control diet was similar to the experimental diets in terms of chemical composition, without *Porphyra* inclusion and comparable to the commercial diets produced by Skretting. A vitamin and mineral premix was added according the Skretting formulation used for commercial trout diets. Yttrium oxide was added at 0.1% (d.w.) as an inert marker for digestibility determination (Carter et al. 2003). Seaweeds were included in diets at different percentages and considering their chemical composition diets were readjusted, mainly with fish meal and wheat starch content.

Digestibility coefficient

Yttrium oxide analyses from faeces were carried out by mass spectroscopy at the CMA Unit at Trinity College, University of Dublin. Apparent digestibility coefficient of dry matter (ADC_{dm}) for the control and test diets was calculated according to Maynard and Loosli (1969) and Cheng and Hardy (2003). Values are presented in Table 3.

$$\text{ADC nutrient}\ (\%) = 100 - [100 \times (\%Y_2O_{3\ \text{diet}}/\%\ Y_2O_{3\ \text{faeces}}) \times (\%\text{nutrient}_{\text{faeces}}/\%\ \text{nutrient}_{\text{diet}})]$$

Carcass analysis

Prior to analysis, carcasses of five fish per tank were homogenised in a blender. Dry matter content was determined by freeze-drying. Samples were frozen at −20°C for 24 h after which they were lyophilised in a freeze-drier (Labconco) for 16 h. Dry samples were placed in a muffle furnace for 14 h at 550°C to determine ash content (AOAC 1997). The Kjeldahl method was used to obtain the crude protein value, multiplying the Kjeldahl nitrogen value by 6.25. Total lipids were analysed gravimetrically after extraction with chloroform–methanol (2:1) as described by Folch et al. (1957).

Statistical analysis

Experimental data were subjected to one-way analysis of variance (ANOVA; α=0.05), using the statistical software package SPSS 14.0. Data were tested for normality (K–S's test), and for homogeneity of variances

(Levene's test) before applying ANOVA. Significant differences between means were compared using a *Post-hoc* LSD test. If significant differences were not found the statistical power was calculated (Searcy-Bernal 1994; Zar 1996). In all statistical tests, $P=0.05$ was taken as the level of significance. All values presented are means± SD (n=3). Initial body weight of all fish ($n=50$) was also included as covariate in the analysis of the morphological body traits and the results discussed at the end (not included).

Results

Biological measurements

Initial body weights (IW) did not present significant differences ($P>0.05$) for any experimental diet when the three tank replicates was used ($n=3$). However, when the total number of fish in tanks ($n=50$) was used, the IW of the *Porphyra* trial did show significant differences between the control and the other diets (data not presented). Final body weight (FW) presented significant lower values ($P<0.05$) for P15 diet (Table 2). Weight gain (WG) did not show significant differences for any experimental diet, *F*-ratio 2.671, power 0.44. Furthermore, the P10 and P15 diet showed a significant lower effect for the hepatosomatic index (HSI) values.

Growth and feed efficiency

Growth factors did not differ significantly between diets ($P>0.05$), with the following power tests; SGR, *F*-ratio 0.786, power 0.15; FCR, *F*-ratio 0.118, power 0.64; and FE *F*-ratio 1.115, power 0.20 respectively. Furthermore, PER did not show any significant differences ($P<0.05$, *F*-ratio 0.292, power 0.08). Due to a mechanical failure some fish died in one of the control and P10 tanks at the second week of the experiment and were excluded from the total. During the trial a few mortalities occurred, fish weight and length were recorded and included in the final tables. ADC_{dm} did not show significant differences between diets, with a power test of F-ratio 1.54, power 0.3. Voluntary feed intake (VFI) increased with seaweed inclusion in diets but not significantly ($P<0.05$, F-ratio 2.234, power 0.38). Protein retention efficiency (PRE) showed a significant lower value for the P15 diet (Table 3)

Carcass composition

Dry matter and lipid content of carcass analysis of rainbow trout fed with *P. dioica*, did not show significant differences between diets ($P>0.05$), whereas ash content showed a significant higher value in the P10 diet compared to the P5 and P15 diets. Carcass protein content for all three experimental diets is higher compared to the control diet of which only the P10 diet was significantly higher ($P<0.05$) to the control (Table 4).

Pigmentation of rainbow trout flesh

Interestingly, when fish were gutted and filleted for analyses, *Porphyra*-fed fish showed a strong pigmentation of the flesh. The coloration increased in intensity from pink orange in the P5 diet to dark orange in the P15 diet. Fish flesh from the control batch was a pinkish-white color. It should be noted that none of the diets contained added astaxanthin.

Discussion

Systematic studies of the use of algae as an ingredient in commercial fish feeds have been few. The results of this study show that inclusion of the red alga *P. dioica* in diets of rainbow trout up to a level of 15% had no significant effect on WG, SGR, FE, PER, and ADC_{dm} (Tables 2 and 3). Nevertheless, final body weights (FW) for the P5, P10 and P15 diets were slightly reduced while P15 differed significantly from the control. The question of whether the FW values were influenced by the difference on IW was answered by including IW as covariate in the statistical

Table 2 Morphological body traits of rainbow trout fed with experimental *Porphyra dioica* diets

	Control	P5	P10	P15
Initial weight (g)	111.86±4.32	106.59±1.97	105.60±5.47	105.26±9.89
Final weight (g)	272.63±8.34^{a}	255.49±6.04^{a}	265.59±4.80^{a}	250.88±15.96^{b}
Final length (cm)	27.08±0.22	26.83±0.39	26.81±0.05	26.55±0.64
Weight gain (g)	160.77±12.26	148.90±5.49	159.99±6.50	145.62±6.62
HSI (%)	1.77±0.09^{a}	1.65±0.10^{ab}	1.51±0.15^{b}	1.49±0.04^{b}
DI (%)	83.74±1.08	82.99±1.69	83.05±0.94	81.90±1.01

Values are the mean±SD of the triplicate mean values. Within a row, means with a letter in common are not significantly different ($\alpha=0.05$)

Table 3 Effect of *Porphyra dioica* on growth performance, feed utilisation and apparent digestibility coefficient of dry matter (ADC_{dm}) in rainbow trout diets

	Control	P5	P10	P15
SGR	1.01±0.08	0.99±0.03	1.05±0.06	0.99±0.04
FCR	0.83±0.04	0.80±0.06	0.83±0.12	0.82±0.02
FE	1.21±0.06	1.25±0.09	1.22±0.16	1.23±0.03
CF	1.38±0.04	1.33±0.05	1.38±0.02	1.34±0.01
PER	2.71±0.14	2.85±0.20	2.70±0.36	2.75±0.07
ADC_{dm}	87.33±2.91	83.99±2.19	80.48±5.92	73.54±9.18
VFI	350.9±17.1	371.7±19.5	366.6±14.5	394.6±35.4
PRE (%)	$63.8±6.8^{a}$	$62.7±6.6^{a}$	$62.6±3.5^{a}$	$56.1±5.1^{b}$
Survival (%)	93.8	86.0	93.6	89.7

Values are the mean±SD of the triplicate groups. Different superscripts on the same row indicate significant differences ($P<0.05$)

analysis. The ANCOVA showed that IW had no significant effect ($P>0.05$) on the FW values for the different diets, indicating that the FW values were only influenced by the diets. Furthermore, this error in the initial distribution of fish did not seem to affect the weight gain (WG) for any of the diets, which would indicate that the growth differences between treatments were maintained. Different results were found by Davies et al. (1997) for *Porphyra purpurea* (Roth) C. Agardh, at inclusion levels of 16.5 and 33% in diets for juvenile thick-lipped grey mullet, *Chelon labrosus* (Risso). They showed that high inclusion levels of *Porphyra* suppressed growth performance and feed utilisation efficiency. Mustafa et al. (1995), showed an increase in body weight and feed utilisations for red sea bream fingerlings, *P. major* fed with three different seaweeds, i.e. *P. yezoensis, A. nodosum* and *U. pertusa* at an inclusion level of 5%. Valente et al. (2006) showed that the green alga *U. rigida* C. Agardh and the red alga *G. bursa-pastoris* (S.G. Gmelin) P.C. Silva can be included at 10% level, and *G. cornea* J. Agardh at a 5% level in fish feed for sea bream with no adverse affects. Furthermore, the hepatosomatic index (HSI), of the P10 and P15 diets were significantly lower ($P<0.05$) to the control diet. It is generally accepted that if these indices are lower than normal values it indicates a change of energy from organ or tissue growth to combat stressors. These indices may vary naturally with food availability, state of sexual maturation and life history (Barton et al. 2002).

Other biological parameters such as, specific growth rate (SGR), food conversion ratio (FCR), feed efficiency (FE) and protein efficiency ratio (PER) did not differ significantly ($P>0.05$) between diets, which would suggest that an inclusion level up to 15% of *Porphyra* in diets for rainbow trout is feasible without affecting these factors (Table 3). Nevertheless, the power of the ANOVA is relatively low, e.g. SGR of *Porphyra* trial, power 0.15, which indicates that there is only a 15% chance of detecting a significant difference at $\alpha=0.05$ for the specific growth rate. Therefore to accept a null hypothesis we would have 85% chance of being wrong (Searcy-Bernal 1994).

Carcass analysis of the *Porphyra* trial did not show large variations, although some of the values were statistically different. Carcass dry matter and lipid content did not present significant differences between diets (Table 4). Increasing the level of seaweed inclusion in feed resulted in higher carcass protein content values than the control. On the contrary, protein retention efficiency (PRE) presented significant lower values for the *Porphyra* inclusion at 15% (Table 3). These results showed that muscle protein deposition was only effected from a 15% *Porphyra* inclusion, which means that up to 10% inclusion would not effect muscle protein deposition. A protein deposition due to an inclusion of seaweeds in diets for rainbow trout could not be concluded from these results. Davies et al. (1997) also reported an increase of crude protein in the carcass of

Table 4 Carcass analyses of rainbow trout fed with four different experimental diets, with *Porphyra dioica* as feed ingredient

	Control	P5	P10	P15
Dry matter (%)	27.47±1.1	26.18±0.8	26.62±1.0	26.85±0.8
Ash (% dm)	$5.4±0.4^{ab}$	$5.0±0.1^{a}$	$5.7±0.1^{b}$	$5.1±0.7^{a}$
Crude protein (% dm)	$67.51±0.6^{a}$	$67.58±0.6^{a}$	$69.37±0.2^{b}$	$68.36±1.4^{ab}$
Lipid (% dm)	25.32±3.8	22.87±1.7	24.78±0.5	23.13±0.9

Mean±SD ($n=5$). Within a row, means with a letter in common are not significantly different ($\alpha=0.05$)

thick-lipped grey mullet with an inclusion level of 16 and 33% of *P. purpurea*, although PRE was not described.

How efficiently feed ingredients are being digested and how much of their nutrient composition can be made available to fish for maintenance and growth is described by the ADC_{dm} (Eusebio et al. 2004). In this study, ADC_{dm} values decreased with increasing percentage of seaweed inclusion, but not significantly $P>0.05$, power 0.3, suggesting that seaweed inclusion might have little effect on growth performance.

The inclusion of *P. dioica* in diets would suggest that an addition of a certain amount of carbohydrates takes place, which was confirmed by the chemical analysis of the feed. However, there is no clear correlation between seaweed inclusion and carbohydrate content in feeds. Seaweed was included at the expense of the wheat starch content affecting most probably the final carbohydrate content in feeds. Soluble carbohydrate content in seaweeds has been reported from 3.7–33.8% (Kaehler and Kennish 1996). Porphyrans, sulphated polysaccharides, are the main carbohydrate components of *Porphyra* spp. which are structurally closely related to agars and agarose (Zhang et al. 2005). These porphyrans are extensively used in the food industry as gelling and stabilizing additives. Certain seaweeds, because of the gelling and binding characteristics of their polysaccharides, have been used as binding agents in pelleted feeds (Hashim and Mat Saat 1992; Jobling 2001).

Several studies have been published describing the effects of inclusion of alternative plant-derived materials. Such fish-feed ingredients are limited by the presence of a wide variety of anti-nutritional substances that interfere with food utilisation and may affect the health and growth of fish (Francis et al. 2001). Nevertheless, only a few such anti-nutrients have been described in seaweeds, such as lectins and tannins (polyphenolic functional groups) that are mainly present in brown seaweeds (Ingram 1985; Ragan and Glombitza 1986) and which may affect protein utilisation and digestion.

The inclusion of vegetable protein in diets for fish has been reported to reduce daily feed intake (Davies et al. 1997). Poor palatability with consequent reduction of voluntary feed intake (VFI) has been given as a reason for poor performance in rainbow trout fed low fish meal and high soy diets (Gomes et al. 1993). This is in contrast with the present study in which an increased voluntary feed intake (VFI) was observed for all diets. The inclusion of *Porphyra* in the diets increased their polysaccharide fiber content and this might have reduced the dietary available energy. As a result, the voluntary feed intake might have increased. It has already been suggested that an increase of VFI is a mechanism for the trout to compensate the possible limiting nutrient deficiency and grow optimally, an attempt by the fish to meet their growth target (Azevedo et al. 2004; Morris et al. 2005). Other studies have reported the presence of dimethyl-β-propiothetin (DMPT) in *Ulva* spp., a chemical substance that operates as a feed attractant for fish (Nakajima 1992; Nakajima et al. 1990).

An observation of considerable interest during this study was the pigmentation of the flesh of the rainbow trout from pinkish-white in the control fish to pinkish-orange to dark orange in the P15 fish. Red algae contain yellow xanthophylls, especially lutein and zeaxanthin which may possibly cause the coloration as these pigments are fat soluble. Pigmentation of the flesh is a major quality attribute for salmonids (De Francesco et al. 2004). Coloration of flesh in salmonids using seaweeds such as *Porphyra* as a natural pigment source may enhance the potential of seaweed inclusion in fish feed and may perhaps replace or reduce artificial colorants currently used by the industry (Nickell and Bromage 1998). Further studies on the advantages and disadvantages of using these pigments in fish feed would be of considerable interest.

This study suggests that *P. dioica* can be included as an ingredient for rainbow trout up to 10% without significant negative effects on growth performance; however, a deeper study would be necessary to consolidate these findings. Future work should focus on the digestibility coefficients of different nutrient classes and on the pigmentation of the flesh. A future design including more tanks would increase the statistical power in order to base conclusions on the effect of the treatment. It would be particularly useful to test *Porphyra* in fish-feed diets for Atlantic salmon in order to research the effects of pigmentation of the flesh as Atlantic salmon is a major economic species with a high value-added compared to trout.

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