Seaweeds as Sustainable Feed Ingredients for Farmed Fish species: Effects on Growth, Immunological Response and Flesh Quality

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Abstract

The potentiality of seaweeds, produced in IMTA systems, as dietary feed ingredients for rainbow trout and Nile tilapia was evaluated. The dietary inclusion of the best performing seaweed in each fish species was further evaluated in juvenile fish, concerning its effects on growth and nutrient utilisation, immunological response and flesh quality. *Gracilaria vermiculophylla* was selected for rainbow trout, whereas in tilapia *Ulva* spp. was selected instead. The inclusion of *Gracilaria* meal in diets for rainbow trout was possible up to 5%, but a higher inclusion level impaired growth. Flesh iodine content doubled in fish fed 5% *Gracilaria* meal, confirming seaweed as a natural and effective tool to increase the nutritional value of rainbow trout. Moreover, in Nile tilapia, the inclusion of *Ulva* spp. meal seems to be possible up to 10% without major effects on growth performance or flesh organoleptic properties, but enhancing the innate immune response of the fish.

Keywords: seaweeds, immunological response, rainbow trout, Nile tilapia

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Introduction

Global population growth and increase in living standards will push up the demand for fish-derived protein in the future. Fish meal has traditionally been the major dietary protein source for fish, but its reduction in aquafeeds is now a priority goal for the further expansion and sustainability of the farmed fish production. On the other hand, the increasing consumer demand for products containing food ingredients from natural sources encourages the use of environmental friendly alternatives to fish meal (FM) able to produce a final product that retains adequate levels of n3 LC-PUFA (Gatlin, Barrows, Brown, Dabrowski, Gaylord, Hardy, Herman, Hu, Krogdahl, Nelson, Overturf, Rust, Sealey, Skonberg, J Souza, Stone, Wilson & Wurtele 2007).

Exogenous feeding in aquaculture unlocks the possibility to tailor fish composition towards increased health-promoting properties, without compromising its sensory attributes and consumer´s acceptance. Over recent years, there has been a renewed interest on the use of seaweeds in aquaculture feeds, not only as a nutrient supply (Valente, Gouveia, Rema, Matos, Gomes & Pinto 2006; Soler-Vila, Coughlan, Guiry & Kraan 2009), but also as a valuable source of bioactive compounds like pigments, vitamins and minerals (Holdt & Kraan 2011; Ribeiro, Gonçalves, Colen, Nunes, Dinis & Dias 2015; Valente, Rema, Ferraro, Pintado, Sousa-Pinto, Cunha, Oliveira & Araújo 2015a). Moreover, seaweeds have remarkably higher concentrations of halogens, rare earth elements and many transition metal elements than terrestrial plants (Hou & Yan 1998). Nevertheless, the protein content of most wild seaweeds is too low to fulfil the nutritional requirements of carnivorous fish. Brown seaweeds are poor protein sources (3–15 % of the dry weight), but red and green seaweeds can have higher protein contents (10–47% of the dry weight) (Fleurence 1999), depending on the season and production conditions.

In recent years, integrated multitrophic aquaculture (IMTA) has been gaining momentum as a way to diminish aquaculture environmental impacts using seaweeds as biofilters in the extractive component of the system (Neori, Chopin, Troell, Buschmann,

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Kraemer, Halling, Shpigel & Yarish 2004). In such ecologically-balanced systems, wastewater from aquaculture effluents is converted into potentially valuable biomass that could be used as an ingredient in fish feed, while reducing the cost of water treatment. Seaweeds produced in IMTA systems usually present higher productivity levels and less variability in protein contents than naturally harvested biomass (Lüning & Pang 2003; Schuenhoff, Shpigel, Lupatsch, Ashkenazi, Msuya & Neori 2003; Mata, Schuenhoff & Santos 2010; Abreu, Pereira, Yarish, Buschmann & Sousa-Pinto 2011).

Previous studies have demonstrated that seaweeds like *Ascophyllum nodosum* (Linnaeus) (Nakagawa, Umino & Tasaka 1997), *Ulva lactuca* (Linnaeus) (Wassef, El Masry & Mikhail 2001), *Gracilaria cornea* (Agardh) and *Gracilaria bursa-pastoris* (Gmelin) Silva (Valente *et al.* 2006), *Porphyra* sp. (Soler-Vila *et al.* 2009), *Macrocystis pyrifera* (Linnaeus) (Dantagnan, Hernández, Borquez & Mansilla 2009) and *Kappaphycus alvarezii* (Doty) Doty ex Silva (Shapawi, Safiin & Senoo 2014) can be used as partial substitutes of dietary fish meal (FM) in aquafeeds. However, the benefits of including seaweeds in fish diets are still in its infancy.

This manuscript summarises the potentiality of seaweeds, produced in IMTA systems, as sustainable feed ingredients in two important farmed fish species: rainbow trout (*Oncorhynchus mykiss*, Walbaum) and Nile tilapia (*Oreochromis niloticus, Linné).*

Material and Methods

A critical aspect when developing diets for fish is the evaluation of their capacity to digest different ingredients. Inclusion of highly digestible ingredients will improve the fish performance whilst reducing the production of wastes. This study evaluated seaweed species selected based on its availability on the Portuguese coast and its potential to be locally produced in integrated multi-trophic aquaculture (IMTA) systems (Pereira, Kraemer, Yarish & Sousa-Pinto 2008; Abreu *et al.* 2011). Three seaweeds, *Porphyra dioica* (J. Brodie & L.M. Irvine), *Ulva* spp. and *Gracilaria vermiculophylla* (Ohmi)

Papenfuss were produced in tanks, in an IMTA system in the facilities of the aquaculture A. Coelho e Castro (Póvoa de Varzim, Portugal, N41°27′10″, W8°46′28″).

The apparent digestibility coefficient (ADC) of the three selected seaweeds was evaluated in rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*). Practical basal mixtures were formulated with the incorporation of 1% chromic oxide (Cr_2O_3) as inert marker. The reference diet consisted of 100% of the basal mixture. For each fish species additional test diets were subsequently produced by mixing 70% of the basal mixture with 30% of each seaweed. Feces were collected using the Choubert System (Choubert, De La Noüe & Luquet 1982).

The dietary inclusion of the best performing seaweed in each fish species was further evaluated in juvenile fish, concerning its effects on growth and nutrient utilisation, immunological response and flesh quality.

Cross sections from the anterior part of the intestine were fixed and embedded in paraffin for light microscopy evaluation of villi height, intestine diameter and muscle layer thickness. The immunological response of fish was evaluated by determining the activity of key components of fish defences: lysozyme, peroxidase and alternative complement pathway (ACH50) as previously described (Araújo, Rema, Sousa-Pinto, Cunha, Peixoto, Pires, Seixas, Brotas, Beltrán & Valente 2015).

Muscle chemical composition and organoleptic properties were evaluated in both rainbow trout and Nile tilapia. Flesh instrumental colour was also evaluated and L*, a* and b* values were recorded. Antioxidant activity of muscle carotenoids were determined by radical scavenging 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods according to Guimarães *et al.* (2007) and Brand-Williams *et al.* (1995).

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Results

Seaweeds impact on nutrient digestibility and fish growth

The ADC´s of seaweeds varied significantly according to both the seaweed and the fish species considered (Pereira, Valente, Sousa-Pinto & Rema 2012). In rainbow trout, protein ADC of *Gracilaria* (88%) was significantly higher than that of *Porphyra sp. (80%) or Ulva* (76%), but in tilapia, protein ADC was higher for *Ulva* (63%) than for the other two seaweeds (51-59%) (Figure 1). Hence, *Gracilaria* sp. was selected for further studies in rainbow trout, whereas in tilapia *Ulva* spp. was selected instead.

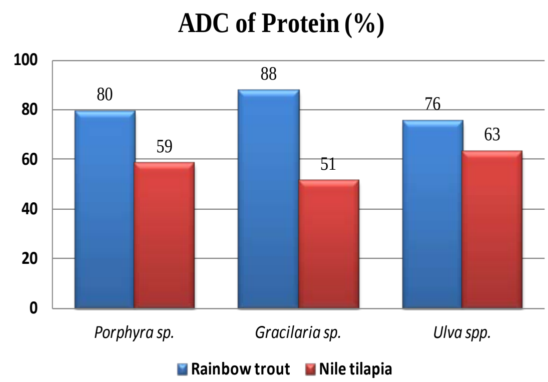

Figure 1. Protein apparent digestibility coefficients (ADC %) of seaweeds in rainbow trout and Nile tilapia (Adapted from Pereira *et al.*, 2012).

Increasing dietary inclusion levels (0, 5 and 10%) of IMTA-cultivated *Gracilaria vermiculophylla* were evaluated in 67 g rainbow trout. Growth and feed efficiency were determined after 91 days at 16 ^{o}C. Although protein intake was similar among groups, the inclusion of 10% *Gracilaria* (G10) induced the lowest protein retention and gain, resulting in the lowest final body weight (Araújo *et al.* 2015). However, the inclusion of 5% *Gracilaria* meal (G5) resulted in similar fish performance and nutrient retention efficiency.

In Nile tilapia juveniles (12g), increasing levels of a mixture of *Ulva* spp. meal (0, 10, 15 and 20%) produced in an IMTA system were evaluated as partial replacement of

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dietary fish meal. After 63 days at 26 ^{o}C, all groups of fish more than tripled their initial body weight. Fish fed 10% *Ulva* meal (U10) had the highest protein efficiency ratio and nitrogen retention efficiency, allowing this fish to growth and reach a final body weight similar to the control fed group (Marinho, Nunes, Sousa-Pinto, Pereira, Rema & Valente 2013). However, higher levels of dietary seaweed impaired growth.

Histological measurements of fish intestinal mucosa showed that seaweeds affected gut morphology and its components. In rainbow trout, G10 diet induced the smallest intestine diameter and lowest *villi* height (Araújo *et al.* 2015). In tilapia, the very same trend was reported with the dietary inclusion of 10% *Ulva*, but without statistical significance (Silva, Valente, Sousa-Pinto, Pereira, Pires, Seixas & Rema 2015).

Seaweed impact on fish immunological response

The immunological response of fish was evaluated by determining the activity of key components of fish defences: lysozyme, peroxidase and alternative complement pathway (ACH50) (Figure 2).

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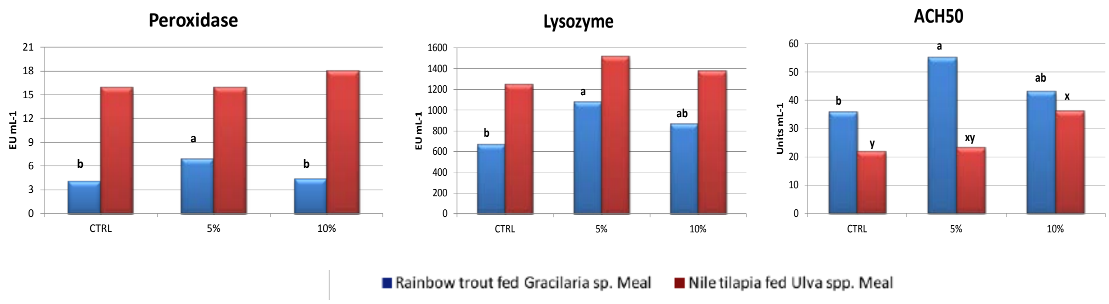

Figure 2. Innate humoral immune parameters of rainbow trout (blue) and Nile Tilapia (red) fed *Gracilaria* sp. meal and *Ulva* spp. meal, respectively (Adapted from Araújo *et al.*, 2015 and Valente *et al.*, 2015b)

In rainbow trout, humoral parameters analysed in plasma of fish fed 5% *Gracilaria* meal (G5) suggest a general and significant stimulation of the immunological system compared to those fed the control diet (Araújo *et al.* 2015).

In Nile tilapia, the peroxidase and lysozyme activities were similar among treatments. The alternative complement pathway increased proportionally to the dietary seaweed inclusion, and tilapia fed U10 showed a significantly higher activity than the control fed fish (Valente, Araújo, Batista, Peixoto, Sousa-Pinto, Brotas, Cunha & Rema 2015b).

Seaweed impact on fish flesh quality

The inclusion of *Gracilaria* meal increased rainbow trout flesh moisture and decreased lipid content, with significant differences between G5 and the control fed fish. Instrumental colour showed that raw and cooked fillets from *Gracilaria*-rich fish were more luminous (L*), less yellowish (b*) and more redish (a*). G10 samples yield the lowest colour intensity (C*), confirming the lowest carotenoid content in these fish (Araújo *et al.* 2015). Moreover, muscle carotenoid extracts presented no significant antioxidant activity through the ABTS and DPPH assays (<20%) (Valente *et al.* 2015a). The sensory evaluation showed that fish fed with seaweed had juicier fillets than the CRT with G5

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presenting the most intense (pinkish) colour. Iodine levels in the flesh increased with the seaweed inclusion, with fish fed G5 doubling its iodine content (214.5 μg/kg) in relation to the CTRL (111.7 μg/kg) (Valente *et al.* 2015a).

In Nile tilapia flesh, colour evaluation of the tristimulus L*, a* and b* indicated significant differences between dietary treatments. The inclusion of 10% *Ulva* spp. meal resulted in fillets with the highest lightness (higher L* value) and yellowness (less negative b*), but the lowest redness (lower a* value). No differences were observed between U5% and the control fed fish. No carotenoids could be found in tilapia muscle. Moreover, sensory attributes showed no significant effects of dietary treatments on visual, olfactory, texture and flavour parameters, with the exception of sour parameter that was lowest in U10-fed tilapia (Valente *et al.* 2015b).

Discussion

The response of fish to dietary seaweed inclusion seems to be dose and species-dependent since nutritional composition and digestibility differ among seaweed species (Pereira *et al.* 2012; Silva *et al.* 2015). In European sea bass (*Dicentrarchus labrax* Linnaeus) juveniles *Gracilaria cornea* could replace dietary fish meal (FM) up to 5%, whereas *G. bursa-pastoris* or *Ulva rigida* could replace 10% FM without compromising growth performance and feed efficiency (Valente *et al.* 2006). The inclusion of *G. vermicullophyla* in rainbow trout diets was shown to be feasible up to 5% with higher levels impairing growth. Moreover, reduced growth observed in G10 could not be associated with poor dietary palatability as food intake was highest in this group, but could be due to the presence of anti-nutritional factors described in plants and algae that can affect the digestion processes.

Modifications in feed ingredients can modulate gut morphology, which is highly responsible for a good digestion and absorption of nutrients. The smallest intestine diameter associated with the lowest *villi* height observed in fish fed 10% *Gracilaria* sp may have reduced absorption surface, contributing to the lowest growth and nutrient retention observed in those fish (Araújo *et al.* 2015). In Nile tilapia, the dietary incorporation of 10 % *Ulva* spp. meal, either produced in IMTA systems (Marinho *et al.* 2013) or wild caught (Güroy, Cirik, Güroy, Sanver & Tekinay 2007; Azaza, Mensi, Ksouri, Dhraief, Brini, Abdelmouleh & Kraïem 2008) did not compromise dry feed intake, growth performance or protein utilisation. This is consistent with recent results from Pereira *et al.* (2012) demonstrating that *Ulva* meal could be considered a practical partial replacement for fish meal in Nile tilapia diets, being better digested than *Gracilaria* or *Porphyra* (Figure 1).

Previous studies showed that algal compounds, mainly polysaccharides such as carrageenan, fucoidan, alginates and β-glucans, modulate the immunological response, and often induce and enhance resistance against infectious diseases, representing primary tools in modern fish farming (Vetvicka, Vannucci & Sima 2013). Lysozyme, peroxidase and complement system are key components of fish defenses since they act against pathogens by directly disrupting their cell walls or through the production of harmful chemicals, such as oxidative radicals (Nayak 2010). However, the immunological response towards dietary seaweed inclusion seems to be dose and species dependent. In rainbow trout the dietary inclusion of 5 % *G. vermiculophylla* meal enhanced fish innate immune response, inducing the highest peroxidase, lysozyme and complement activities (Araújo *et al.* 2015), but in Nile tilapia, the dietary inclusion of *Ulva* spp.meal had no beneficial effect on lysozyme or peroxidase activities (Valente *et al.* 2015b). The complement activity (ACH50) in Nile tilapia increased concomitantly to the dietary inclusion level of *Ulva* spp. meal, reaching maximal activity with the highest seaweed inclusion level (U10). Similarly, supplementation of 5 % *Ulva* spp. meal in the diet for red sea bream (*Pagrus major*, Temminck & Schlegel) enhanced complement activity and disease resistance without impairment of growth (Satoh, Nakagawa & Kasahara 1987). This suggests that dietary inclusion of seaweeds initiates activation of fish innate defence mechanisms, which may be due to its high content of carbohydrates. El-Boshy *et al.* (2010) have previously reported immunostimulant properties of both β-glucan and laminaran in farmed Nile tilapia, suggesting its use under immune depressive stressful condition to increase their resistance to diseases. The main polysaccharide in *Gracilaria* spp. is agar, with similar structural and functional proprieties to carrageenan, but other compounds such as β-carotene, may also modulate fish humoral immune response. Studies evaluating the effects of such compounds in fish immune response are scarce. Although both *Gracilaria*- and *Ulva*-rich diets seems to influence innate immune system in rainbow trout and tilapia, respectively, only further experiments exposing fish to stress conditions could confirm their capacity to enhance fish immunity and disease resistance.

The nutritional modulation of flesh quality traits has been considered in several fish species as an effective way of enhancing the fillet quality improving its nutritional value for human consumption. Seaweeds present a valuable content of micronutrients, such as carotenoids, vitamin E and minerals (Holdt & Kraan 2011). One of the most important minerals in seaweeds is iodine, a halogenated trace element that is essential for growth and metabolism since it is involved in thyroid hormone synthesis in humans and animals. The dietary inclusion of 5% *G. vermiculophylla* in diets for rainbow trout resulted in a two-fold increase of fillet iodine content, confirming this seaweed species as a natural and effective tool to increase the nutritional value of rainbow trout (Valente *et al.* 2015a). Moreover the dietary inclusion of *Laminaria* sp. was shown to be a valuable way of increasing fillet iodine content in both freshwater (Schmid, Ranz, He, Burkard, Lukowicz, Reiter, Arnold, Le Deit, David & Rambeck 2003) and marine fish species (Ribeiro *et al.* 2015). In gilthead seabream *L. digitata*, an iodine-rich macroalgae, was an effective and natural strategy to fortify muscle with iodine, showing that a 160 g portion of steam-cooked fillets could cover approximately 80% of the Daily Recommended Intake for iodine and 370% of the Daily Adequate Intake of EPA+DHA for enhanced cardiovascular health in adults (Ribeiro *et al.* 2015).

In rainbow trout, the sensory panel perceived fillets from fish fed with 5% *Gracilaria* meal (G5) as the sample with higher colour intensity and juicer than those fed the control. Nevertheless, G5 could not improve flesh carotenoid deposition nor improve fillet preservation (Valente *et al.* 2015a). Previous studies demonstrated that the flesh

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dominant carotenoids in Nile tilapia are astaxanthin and canthaxanthin (Czeczuga, Czeczuga-Semeniuk, Káyszejko & Szumiec 2005). However, the absence of these two pigments in the *Ulva* spp. meal (Sefc, Brown & Clotfelter 2014) may explain the lack of detectable carotenoids in muscle of tilpia either fed 5 or 10% *Ulva* meal (Valente *et al.* 2015b), as cichlid like other fish cannot synthesise them. Moreover, the incorporation of 30 % *Sargassum siliquastrum* in diets for Silver seabream (*Sparus sarba*) significantly increased flesh total bromophenol content providing the desirable sea-like flavour (Ma, Chung, Ang & Kim 2005). But, the dietary inclusion of *Ulva* meal resulted in no major visual, olfactory, texture or flavour parameters in tilapia fillets (Valente *et al.* 2015b).

In conclusion, the inclusion of *Gracilaria* meal in diets for rainbow trout is possible up to 5%, but a higher inclusion level impairs growth. However, in Nile tilapia, the inclusion of *Ulva* spp. meal seems to be possible up to 10 % without major effects on growth performance or flesh organoleptic properties, but enhancing the innate immune response of the fish.

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