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Iodine enrichment of rainbow trout flesh by dietary supplementation with the red seaweed *Gracilaria vermiculophylla*

Luísa M.P. Valente [a,b,*], Paulo Rema [c], V. Ferraro [d], M. Pintado [d], Isabel Sousa-Pinto [a,e], Luís M. Cunha [f], M.B. Oliveira [g], Mariana Araújo [a,g]

[a] CIIMAR/CIMAR — Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal
[b] ICBAS — Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[c] CECAV/UTAD — Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, Apartado 1013, 5001-801 Vila Real, Portugal
[d] CBQF — Centro de Biotecnologia e Química Fina, Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Centro Regional do Porto, Rua Arquitecto Lobão Vidal, Apartado 2511, 4202-401 Porto, Portugal
[e] FCUP — Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal
[f] LAQV, REQUIMTE, Faculdade de Ciências, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, 7, 4485-661 Vila do Conde, Portugal
[g] LAQV, REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

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ABSTRACT

The effects of different dietary inclusion levels (0, 5 and 10%) of IMTA-cultivated *Gracilaria vermiculophylla* on rainbow trout growth performance and flesh quality traits were evaluated. Flesh chemical composition (moisture, protein, lipid, vitamin E and iodine contents), sensory attributes and instrumental color were determined after a feeding period of 91 days with the experimental diets. The antioxidant activity of muscle carotenoids and hydrolysates was also determined by the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. By the end of the experiment, fish fed CTRL or G5 diet reached similar body weight (215–220 g), but fish fed G10 weighed significantly less (167 g). Seaweed inclusion increased flesh moisture and decreased lipid content, with significant differences between the G5 and CTRL groups. Vitamin E content varied among treatments with CTRL fed fish presenting the highest levels of α-tocopherol. 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). Instrumental color showed that cooked fillets from fish fed seaweed-rich diets were more luminous (L*), less yellowish (b*) and more reddish (a*) than the CTRL. The sensory evaluation showed that fish fed with seaweed had juicier fillets than the CTRL with G5 presenting the most intense (pinkish) color. The instrumental differences are balanced in such a way that the sensory panel perceived G5 as the sample with higher color intensity. Muscle carotenoid extracts presented no significant antioxidant activity through the ABTS and DPPH assays, whereas muscle hydrolysates showed similar antioxidant activity in all dietary treatments (ca. 33% through DPPH assay).
The inclusion of *Gracilaria* sp. meal in diets for rainbow trout seems to be possible for up to 5%, as higher inclusion levels resulted in significantly smaller fish. The sensory panel perceived G5 as the sample with higher color intensity and juicier than the CTRL. Moreover, flesh iodine content doubled in fish fed G5, confirming seaweed as a natural and effective tool to increase the nutritional value of rainbow trout.

Statement of relevance/impact of your paper to the general field of commercial aquaculture

This paper is relevant for the aquafeed industry and fish consumers. 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 farmed fish production. Seaweeds may act as nutrient supply in fish, which in turn could ultimately function as vehicle of valuable compounds in human nutrition. This paper shows that 5% *Gracilaria* sp. meal can be a natural and effective tool to increase the nutritional value of rainbow trout flesh without impacting growth.

* Corresponding author at: CIIMAR/CIMAR — Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas, 289, 4050-123 Porto, Portugal.
E-mail address: lvalente@icbas.up.pt (L.M.P. Valente).

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1. Introduction

Rainbow trout (*Oncorhynchus mykiss*) is widely produced around the world and is the second most consumed fish species in Europe (FAO, 2013). It is a carnivorous fish species that requires 35 to 45% of dietary protein (NRC, 2011). 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. Some encouraging results in salmonids were obtained by partial and even total replacement of dietary fish meal protein with plant protein (PP) without deleterious effects on growth performance (Espe et al., 2006, 2007; Kaushik et al., 1995). However, the inclusion of high PP levels in fish feeds can have variable consequences on flesh quality traits and organoleptic characteristics (de Francesco et al., 2004; Pratoomyot et al., 2010). So, the aquaculture industry has to create economically viable and environmentally friendly alternatives to fish meal (FM) able to produce a final product that retains the adequate levels of flesh essential fatty acids (Gatlin et al., 2007; Hardy, 2010; Pratoomyot et al., 2010) without compromising the sensory attributes and hence the consumer's acceptance of the fish. Moreover, the increasing consumer demand for products containing bioactive food ingredients from natural sources encouraged the use of seaweeds as a dietary supplement in aquafeeds, not only as a nutrient supply, but also as a valuable source of bioactive compounds like pigments, vitamins and minerals (Holdt and Kraan, 2011). Hence, seaweeds may act as a nutrient supply in fish, which in turn could ultimately function as a vehicle of valuable compounds in human nutrition.

Previous studies demonstrated that the dietary inclusion of red seaweeds *Gracilaria cornea*, *Gracilaria bursa-pastoris* and *Porphyra* spp. can be used as partial substitutes of dietary FM without compromising growth or feed efficiency in several fish species (Marinho et al., 2013; Silva et al., in press; Soler-Vila et al., 2009; Valente et al., 2006), but, so far the effects of dietary inclusion of seaweeds on flesh quality traits have been poorly evaluated in farmed species. *Gracilaria* sp. presents a valuable content of micronutrients, such as carotenoids, vitamin E and minerals (Holdt and Kraan, 2011) able to modulate fish flesh quality, improving its nutritional value. Carotenoids exhibit antioxidant activity due to its ability to scavenge singlet oxygen and other free radicals, whereas vitamin E stabilizes peroxyl radicals, contributing to minimize lipid oxidation during fish storage (NRC, 2011). Vitamin E is an essential nutrient in salmonid diets and the form with the highest biopotency is α-tocopherol followed by β- and γ-tocopherols (Hamre, 2011; Parazo et al., 1998). Due to its antioxidant properties, the accumulation of vitamin E in the fillet performs an important role in protection against cell injuries mediated by free-radicals *in vivo*, and may also minimize lipid oxidation *post-mortem* following processing and storage (Frigg et al., 1990; Kamireddy et al., 2011; Parazo et al., 1998). Synthetic carotenoids and vitamin E, mainly in the form of α-tocopheryl acetate, are commonly used in aquafeeds for salmonids (Akhtar et al., 1999), but the dietary inclusion of seaweed is emerging as a cost-effective solution to the use of synthetic forms contributing to both muscle pigmentation and fillet preservation (Soler-Vila et al., 2009). Moreover, Le Tutour (1990) reported a synergistic effect of the antioxidant activities of marine algal extracts with vitamin E. Marine algae are a rich source of antioxidants, although its radical scavenging activity is dose-dependent (Chan et al., 2014; Kuda et al., 2005) and also depends on species, processing and storage conditions (Farvin and Jacobsen, 2013; Jiménez-Escrig et al., 2001; Kuda et al., 2005; Matsukawa et al., 1997; Yildiz et al., 2011).

Seaweeds are also rich sources of minerals and have remarkably higher concentrations of halogens, rare earth elements and many transition metal elements than those of terrestrial plants (Hou and Yan, 1998). 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. Iodine deficiency leads to the appearance of hypothyroidism and goiter in vertebrates, including fish (Lall, 2002; Leatherland, 1994; Ribeiro et al., 2012). According to NRC (2011) the requirement of salmonids for dietary iodine is relatively low (1.1 mg/kg), but iodine deficiency might still occur in freshwater systems with low iodine content or in closed tank systems where ozonation of artificial seawater may alter the relative concentrations of iodine species (Ribeiro et al., 2012; Sherrill et al., 2004). Iodine deficiency disorders (IDD) are described in many geographic areas due to iodine scarcity. Therefore, the enrichment of iodine along the food chain is a valuable strategy to increase the consumers' daily iodine intake preventing deficiencies of this mineral in humans. In Atlantic salmon, the dietary supplementation of an iodine salt was shown to increase fillet iodine content threefold (Julshamn et al., 2006); a single meal of a 200 g of such fillet was enough to cover the recommended daily iodine intake of 150 μg/g (WHO, 2007). The highest naturally occurring iodine content is in seafood. Brown seaweeds, such as *Laminaria* and *Saccharina*, have the highest iodine content, reaching 1200 mg/100 g of dry matter, followed by red seaweeds like *Gracilaria* sp. (426 mg/100 g) (Holdt and Kraan, 2011; Wen et al., 2006). The dietary inclusion of *Laminaria* sp. was shown to be a valuable way of increasing fillet iodine content in both freshwater (Schmid et al., 2003) and marine fish species (Ribeiro et al., 2015), but other seaweeds also merit further consideration as natural sources of iodine.

The objective of this study was to evaluate growth performance and flesh quality traits of rainbow trout fed increasing levels (0, 5 and 10%) of IMTA-cultivated *Gracilaria vermiculophylla*. Flesh chemical composition (moisture, protein, lipid, vitamin E and iodine), sensory attributes and instrumental color were determined after a feeding period of 91 days with the experimental diets. The antioxidant activity of muscle carotenoids and hydrolysates was determined by the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods.

2. Material and methods

2.1. Experimental diets

G. vermiculophylla was raised in an IMTA system according to Abreu et al. (2011). The seaweed was collected and dried for 48 h, in an oven at 50 °C, and grinded to 1.5–2 mm size particles. A control diet (CTRL) was formulated according to the nutritional requirements of rainbow trout (*O. mykiss*) and compared with two test diets (G5 and G10), where fish meal was replaced by 5% and 10% of *Gracilaria* sp. meal (Table 1). All diets were extruded (SPAROS, Lda) by means of a pilot-scale twin-screw extruder Clextral BC45 (Clextral, France) with a screw diameter of 55.5 mm and temperature ranging 105–110 °C (pellet size 2.5 mm). Diets were dried in a convection oven (OP 750-UF, LTE Scientifics, United Kingdom) for 2 h at 60 °C. Pellets were allowed to cool at room temperature, and subsequently the oil was added under vacuum coating conditions in a vacuum mixer (PG-10VCLAB, Dinnisen, The Netherlands).

2.2. Growth trial

Experiments were performed by trained scientists and following the European Directive 2010/63/EU of the European Parliament and of the Council of European Union on the protection of animals used for scientific purposes.

Rainbow trout (*O. mykiss*) were reared in tanks at the Experimental Research Station of University of Trás-os-Montes e Alto Douro (UTAD, Portugal). Nine homogeneous groups of 25 fish (average body weight 67.0 ± 0.4 g) were distributed among nine fiber-glass tanks (volume 120 L, water flow rate: 120 L h^{-1}), in a flow-through freshwater system (temperature: 16 ± 1 °C) exposed to natural photoperiod. The experimental diets were randomly assigned to the tanks establishing triplicate groups of fish per treatment that were hand-fed twice daily until apparent visual satiety for 91 days.