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Natural fortification of trout with dietary macroalgae and selenised-yeast increases the nutritional contribution in iodine and selenium

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ABSTRACT

Fish and seafood consumption are increasing worldwide and the contribution of aquaculture products to consumers' diets is significant. External feeding in aquaculture unlocks the possibility of tailoring fish products with health beneficial compounds. A study was undertaken to evaluate the feed fortification with an iodine-rich macroalgae (*Laminaria digitata*) and selenised yeast, at its maximum permitted levels, on minerals and vitamins content in rainbow trout edible part. Dietary supplementation resulted in a six-fold increase for iodine and a 2.9-fold increase for selenium contents in trout fillets without altering sensorial traits. The fortified fish presented a nutritional contribution of 12.5% DRI for iodine and 78% DRI for selenium, but all produced fish could supply 80% DRI for vitamin D_3. Overall, fish from this trial could be labelled as "high in selenium and high in vitamin D_3" under the EFSA definition for a functional food.

1. Introduction

Fish and shellfish are generally associated with healthy dietary patterns and improved wellbeing. Seafood is a valuable source of multiple essential nutrients, since most species provide the recommended amounts of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA), contributing also to cover the needs of other essential nutrients, such as vitamin D, iodine or selenium (European Food Safety Authority EFSA, 2014a). In recent years, the identification of functional bioactive nutrients from marine origin and their biological effects has been the object of important research efforts (Shahidi & Ambigaipalan, 2015). High fish consumption, with high n-3 LCPUFA levels, namely eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) fatty acids, has been unequivocally associated to a protective role against a number of human diseases (FAO/WHO, 2011; Raatz, Silverstein, Jahns & Picklo, 2013). Other essential nutrients such as selenium, iodine and vitamin D, of which fish is considered the main dietary source have also been positively correlated with disease prevention and improved health status. Additionally, recent studies suggest that some health benefits, attributed to LCPUFA, may be potentiated in the presence of selenium (Berr et al., 2009) and iodine (Bath, Steer, Golding, Emmett & Rayman, 2013). These outcomes reinforce the importance of eating fish for its holistic properties rather than the ingestion of pills or supplements. A food-based approach has also been recommended by nutritionists for achieving nutrient adequacy, preventing and treating diseases. Dietary guidelines recommend at least two fish portions per week with cardiovascular protective effects and up to four servings per week during pregnancy with better functional outcomes of neurodevelopment in children (EFSA, 2014a).

Worldwide seafood consumption is steadily expanding, with per capita records of 20 kg in 2014, and prospects are to continue rising in the next years (FAO, 2015). Provision of seafood from capture fisheries is declining though aquaculture is overcoming this supply issue and the actual contribution at world level already overtook that of wild fish for human consumption (FAO, 2015). However, the current trend in aquafeeds for replacing marine-derived ingredients (fish meal and fish oil) by vegetable protein and oil sources can interfere with the fish nutritional profile. For instance, vegetable ingredients are often characterized by low amounts of n-3 LCPUFAs. Although conditioned by the elemental soil content of cultivars, cereals, pulses and oilseeds are also poorer sources of iodine and selenium when compared to marine protein ingredients (Van Paemel, Dierick, Janssens, Fievez & De Smet, 2010). Moreover, current levels of vitamins and minerals in fish feeds target an optimal fish growth and welfare, without regarding a potential enhancement of the beneficial effects to consumers' health. Within this scenario, a new perspective towards consumers' dietary

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needs must be considered when designing aquafeeds. Fillets traits have already been effectively changed by modulating fish feeds in terms of bioactive fatty acids (Kennedy, Bickerdike, Berge, Dick & Tocher, 2007; Ramos et al., 2008; Rosa, Andrade, Bandarra & Nunes, 2010), selenium (Lorentzen, Maage & Julshamn, 1994; Schram, Schelvis-Smit, Van Der Heul & Luten, 2010) and iodine (Julshamn, Maage, WaagbØ, & Lundebye, 2006; Ramalho Ribeiro et al., 2015). However, the efficacy of muscle fortification seems to be dependent on supplemental dose, feeding strategies and also important the supplement product form (inorganic vs. organic). Additionally, some of these studies also lack an overall assessment regarding the nutritional contribution of fish to human consumption.

A dietary fortification trial was performed with rainbow trout owed to its importance as a major freshwater species consumed in Europe. The macroalgae *Laminaria digitata* and selenium-enriched yeast were used as iodine and selenium sources and their effects on fish fortification were evaluated, either isolated or concomitantly. Afterwards, the nutritional contribution was estimated and sensorial traits of trout fillets were also assessed.

2. Material and methods

2.1. Experimental diets

Four experimental diets were formulated to be isonitrogenous (42.7% crude protein), isolipidic (23.3% crude fat) and isoenergetic (23.5 MJ·kg^{-1} gross energy) (Table 1). A control diet (CTRL), similar to a commercial trout feed was formulated to contain 1.9 mg iodine·kg^{-1} supplied as potassium iodide and 0.25 mg selenium·kg^{-1}, supplied as sodium selenite through the mineral premix and the endogenous content of the various ingredients. Based on this control formulation, three other experimental diets were manufactured. The LAM diet targeted an iodine level of 20 mg·kg^{-1}, supplied as *Laminaria digitata*, an iodine-rich macroalgae; SE diet targeted a total selenium level of 0.5 mg·kg^{-1}, supplied partially as selenium enriched yeast and LAMSE diet with a simultaneous supplementation of 20 mg iodine·kg^{-1}, as *Laminaria digitata*, and of 0.5 mg selenium·kg^{-1}, supplied as selenised yeast. The levels of 20 mg iodine·kg^{-1} and 0.5 mg selenium·kg^{-1} were adopted since it is the currently authorized maximum content of total iodine and selenium in complete feeds for fish in the European market. Experimental extruded diets were manufactured at SPAROS Lda (Olhão, Portugal).

2.2. Growth trial and sampling

The trial was conducted at the Experimental Research Station of University of Trás-os-Montes e Alto Douro (UTAD, Portugal). Experiments were directed by trained scientists (following category C FELASA recommendations) and in compliance with the European (Directive 2010/63/EU) and Portuguese (Decreto-Lei n°. 113/2013, de 7 de Agosto) legislation on the protection of animals for scientific purposes. UTAD facilities and their staff are certified to house and conduct experiments with live animals ('group-1' license by the 'Direção Geral de Veterinária', Ministry of Agriculture, Rural Development and Fisheries of Portugal).

Eight homogenous groups of 48 rainbow trout each, with a mean initial body weight of 238 g were stocked in 350 L fiberglass tanks, supplied with flow-through freshwater (water-flow rate: 7 L·min^{-1}, temperature: 15 ± 1 °C) and exposed to natural photoperiod conditions (14 light/10 h dark). Each experimental treatment was tested in duplicate tanks over 91 days. Fish were hand-fed to apparent satiety, twice a day. Final samplings were done 48 h following the last meal and fish were slaughtered by immersion in ice-water slurry (4:1) until death. At the start (6 fish from the initial stock) and at the end of the trial, three fish from each tank were sampled for analysis of whole-body composition. Moreover, trout skinless fillets were collected at the start (n = 3) and at the end of trial (n = 2 pools of 3 fish each), stored at −80 °C for analysis of minerals and vitamins content and fatty acid composition. For sensory analysis, ten fish from each treatment were weighed, scaled, filleted and kept at 4 °C until sensory assessment.

Table 1
Ingredients and proximate composition of experimental diets.

Ingredients, g·kg^{-1}	CTRL	LAM	SE	LAMSE
Fishmeal LT 70[a]	125.00	125.00	125.00	125.00
Fishmeal 60[b]	50.00	50.00	50.00	50.00
Soy protein concentrate[c]	160.00	160.00	160.00	160.00
Wheat gluten[d]	100.00	100.00	100.00	100.00
Corn gluten[e]	100.00	100.00	100.00	100.00
Soybean meal[f]	50.00	50.00	50.00	50.00
Rice protein concentrate[g]	50.00	50.00	50.00	50.00
Wheat meal	121.30	117.65	121.20	117.55
Fish oil[h]	195.00	195.00	195.00	195.00
Vitamin & mineral premix[i]	10.00	10.00	10.00	10.00
Soy lecithin	2.00	2.00	2.00	2.00
Guar gum	4.00	4.00	4.00	4.00
Zeolite	10.00	10.00	10.00	10.00
Antioxidant	2.00	2.00	2.00	2.00
Dicalcium phosphate	11.40	11.40	11.40	11.40
Astaxanthin	0.30	0.30	0.30	0.30
L-Lysine	7.00	7.00	7.00	7.00
DL-Methionine	2.00	2.00	2.00	2.00
Macroalgae (Laminaria)[j]		3.65		3.65
Selenised yeast[k]			0.10	0.10
Dry matter (DM), %	95.2 ± 0.1	95.7 ± 0.1	96.0 ± 0.2	95.8 ± 0.0
Crude protein, %DM	42.7 ± 0.1	42.6 ± 0.4	42.7 ± 0.0	42.8 ± 0.1
Crude fat, % DM	23.3 ± 0.1	23.4 ± 0.2	23.4 ± 0.1	23.4 ± 0.1
Ash, % DM	8.5 ± 0.0	8.8 ± 0.1	8.7 ± 0.1	8.8 ± 0.1
Gross energy, MJ·kg^{-1} DM	23.5 ± 0.1	23.6 ± 0.2	23.5 ± 0.1	23.5 ± 0.0
Total phosphorus, % DM	1.13 ± 0.02	1.21 ± 0.03	1.14 ± 0.02	1.20 ± 0.01
Iodine, mg·kg^{-1} DM	1.89 ± 0.11	22.70 ± 1.49	2.09 ± 0.06	22.35 ± 3.13
Selenium, mg·kg^{-1} DM	0.24 ± 0.02	0.26 ± 0.02	0.53 ± 0.02	0.51 ± 0.01

[a] Peruvian fishmeal LT: 71% crude protein (CP), 11% crude fat (CF), EXALMAR, Peru.
[b] Fair Average Quality (FAQ) fishmeal: 62% CP, 12%CF, COFACO, Portugal.
[c] Soycomil P: 65% CP, 0.8% CF, ADM, The Netherlands.
[d] VITEN: 85.7% CP, 1.3% CF, ROQUETTE, France.
[e] Corn gluten meal: 61% CP, 6% CF, COPAM, Portugal.
[f] Solvent extracted dehulled soybean meal: 47% CP, 2.6% CF, SORGAL SA, Portugal.
[g] Rice 50: 48.3%CP, 6.5% CF, SEAH International, France.
[h] COPPENS International, The Netherlands.
[i] Premix for marine fish, PREMIX Lda, Portugal. Vitamins (IU or mg·kg^{-1} diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20,000 IU; DL-cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 1000 mg; inositol, 500 mg; biotin, 3 mg; calcium pantothenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg·kg^{-1} diet): cobalt carbonate, 0.65 mg; copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate,7.5 mg; sodium chloride, 400 mg; calcium carbonate, 1.86 g; excipient wheat middlings.
[j] Dry *Laminaria digitata*: 5.4% CP, 0.5% CF, 3700 mg iodine·kg^{-1}, Agrimer, France.
[k] ALKOSEL R397: 2200 mg selenium·kg^{-1}, Lallemand, France.

2.3. Analytical methods

Proximate composition analysis of the diets and whole fish was made by the following procedures: dry matter by drying at 105 °C for 24 h; ash by combustion at 550 °C for 12 h; crude protein (N × 6.25) through the release of nitrogen by a combustion technique followed by thermal conductivity detection (LECO FP528, Leco Instruments, USA); crude fat after dichloromethane (CID: 6344) extraction by the Soxhlet method; total phosphorus in the feeds was quantified according to the ISO/DIS 6491 method using the vanado-molybdate reagent; and gross energy in an adiabatic bomb calorimeter (model C2000, IKA-Werke GmbH & Co. KG, Staufen, Germany). Minerals (Fe, Zn, Mg, K, I and Se) content in feeds and fillets was determined at an external