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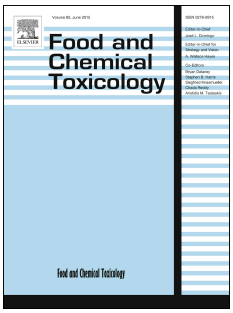

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Author contributions

B.K.L., K.G., J.D., G.A., A.M. were responsible for the design and performance of the trout trial including seaweed and feed preparation. H.A., B.K.L., J.J.S., K.G. were responsible for the chemical analyses. L.M.P.V. and V.S. were responsible for the intestinal histological evaluation. K.G., B.K.L., H.A. L.M.P.V wrote the manuscript. All authors provided comments to the manuscript.

Growth performance, bioavailability of toxic and essential elements and nutrients, and biofortification of iodine of rainbow trout (*Onchorynchus mykiss*) fed blends with sugar kelp (*Saccharina latissima*)

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Aquaculture production is demanding novel feed ingredients that reflect natural marine nutrient levels, that are also essential to humans. In this regard, biofortification through addition of iodine-rich sugar kelp in feed formulations was assessed in a 12 week rainbow trout trial. Yttrium inclusion in feed allowed determinations of apparent absorption coefficients of essential and potentially toxic elements and apparent digestibility coefficient of nutrients. E.g. apparent absorption coefficients in trouts fortified feed with 1-4% dw kelp were 67-61% As, 32-40% Cd, <5% Fe; 80-83% I; 66-58% Se. Iodine concentrations in feed up to 239 mg/kg (~4% kelp) was proportional to iodine accumulation in trout fillets (R^2=1.00) with 0.5% transfer ratio. Feed iodine concentrations up to 117 mg/kg (~2% kelp) did not affect growth performance negatively, but increased significantly protein efficiency ratio after eight weeks feeding. However, 4% kelp meal inclusion affected final growth and hepato somatic index, and caused histomorphological changes in the intestine. All fillets had low toxic element concentrations (As, Cd, Hg, Pb). The potential applicability of *Saccharina latissima* as feed ingredient to tailor iodine concentration in farmed fish is evident. Consuming of a 160 g fillet (2% kelp) contributes ~60% of recommended daily iodine intake for adults.

1

Abbreviations

AAC apparent absorption coefficient
ADC apparent digestibility coefficient
As arsenic
BM biomass
Ctr control
Cd cadmium
CF condition factor
CRM certified reference material
DHA docosahexaenoic acid
dw dry weight
EHA eicosapentanoic acid
FCR feed conversion ratio
Fe iron
Hg mercury
HSI hepato somatic index
iAs inorganic arsenic
AsIII arsenite
AsV arsenate
IMTA integrated multi-trophic aquaculture
ICP-MS inductively coupled plasma mass spectrometry
Pb lead
PER protein efficiency ratio
PUFA, polyunsaturated fatty acids
NFE nitrogen free extractives
Se selenium
SGR specific growth rate
TMAH tetramethylammonium hydroxide
ww wet weight
Y yttrium
Zn zinc

2

3

4 **1. Introduction**

5 Global seafood consumption *per capita* has increased steadily from 9 kg in 1961 to more than 20 kg in
6 2015, surpassing global consumption of meat (FAO, 2018), in 2015 accounting for 17% of animal protein
7 consumed worldwide (FAO, 2018).

8 Aquaculture products comprise half of all seafood consumed, and this industry is expected to be the main
9 supplier of fish and shellfish in the future (FAO, 2018). Aquaculture depends on natural feed resources,

and with increased production, traditional marine ingredients, such as fish meal and fish oil are being replaced. For example, in the Norwegian Atlantic salmon farming, fish meal and fish oil decreased from 90% to 30% between 1990 and 2013. Thus, farmed Atlantic salmon has become a net producer of marine protein using only 0.7 kg marine protein to produce 1 kg salmon protein (Ytrestøyl et al., 2015).

Novel ingredients should provide fish products with the same high nutritional value to promote the associated health benefits, previously described for fish. Wild oily/fatty fish are known to be rich sources of beneficial components, such as ω3-polyunsaturated fatty acids and vitamin D. Vitamin D is e.g. found in Atlantic salmon and rainbow trout at 1.6±0.5 and 5.0±2.3 µg/100 g, respectively (Jakobsen and Smith, 2017). However, it is important to consider whether the benefits of fish composition are compromised by feed substitutions, as reported by Spargue et al. (2016).

Wild fish are also a good dietary source of essential minerals such as selenium and iodine. Iodine typically occurs at low concentrations in most foodstuffs with marine fish, especially lean fish, containing the highest concentrations (18-1210 µg/100 g; Nerhus et al., 2018).

Iodine is essential for synthesis of thyroid hormones, which depends on an adequate and regular supply of iodine, and regulate many functions in the body, including metabolism, energy production, mood and neural development. According to WHO, globally iodine deficiency is the greatest cause of preventable brain damage in childhood and they recommend a daily iodine intake of 150 µg for adults (WHO, 2004). Pregnant women, in particular, need more iodine to ensure thyroid hormones are available for the developing fetus. Inadequate maternal iodine status during pregnancy is associated with later impairment of language skills in their children at the ages of 6, 12 and 18 month (Markhus et al., 2018) and impacts the cognitive outcome of children aged eight years (Bath et al., 2013). Thus, it is not only iodine deficiency but also insufficiency that can have detrimental effects on the health of their children.

To prevent iodine deficiency, many countries have fortified salt with iodine. However, health recommendations to limit salt intake may reduce iodine contribution from salt intake. As iodine is a

natural mineral present in the marine environment and occurs naturally in fish, it is obvious to consider farmed fish as a candidate foodstuff to become biofortified with iodine, e.g. by using iodine-rich feed ingredients as certain types of seaweed. Macroalgae, especially brown algae species contain high concentrations of iodine, some above 10 g iodine/kg dry weight (dw) (Schiener et al., 2015), such as sugar kelp (*Saccharina latissima)* and oarweed (*Laminaria digitata*). Both species are produced for a variety of purposes, e,g. *Saccharina latissima* is grown on longlines (robe), as part of integrated multitrophic aquaculture (IMTA) to capture nutrients around salmon cages and a few studies have reported fortification using macroalgae (*Gracilaria vermiculophylla, Laminaria digitata*) in fish (rainbow trout, gilthead seabream) to increase iodine content (Valente et al., 2015, Ribeiro et al., 2015, 2017).

In addition to the beneficial essential elements in fish and macroalgae, they may also contain elevated levels of potential toxic elements like arsenic, cadmium, lead and mercury. Arsenic exists as both organic and inorganic compounds, of which inorganic arsenic is the most toxic form of arsenic and recognized as a class I human carcinogenic (IARC, 2011). In some types of seaweed inorganic arsenic may be present at relatively high concentrations (Duinker, 2014). In contrast, only very low concentrations commonly occur in wild caught and farmed fish species (Julshamn et al, 2012). Mercury is known to accumulate in the marine food webs and consequently the highest concentrations are reported in large predatory fish such as tuna or swordfish (Storelli et al., 2005). Cadmium and lead are typically found at low concentration in most fish species but for seaweed and shellfish higher concentrations of cadmium can be found (Maulvault et al., 2015, Rasmussen et al., 2017).

The aim of the present study was to evaluate a novel natural feed ingredient, sugar kelp macroalgae *Saccharina latissima,* for use in rainbow trout (*Onchorynchus mykiss*) diets. Feed formulations with 1%, 2% and 4% dried sugar kelp (w/w) were fed to rainbow trout in a controlled trial to determine the most appropriate level for future aquaculture production. The controlled trial assessed several growth performance parameters, as well as impacts on apparent digestibility/absorption of nutrients and essential and toxic elements from the experimental feeds, and fillet biofortification.

2. Material and methods

2.1. Animal husbandry

Rainbow trout (all female), were obtained from a commercial fish farm (Lundby Fisk, Nibe, Denmark) and transported to DTU Aqua facilities at the North Sea Research Centre, Hirtshals, Denmark. Initially, they were kept in large (3,000 L) circular outdoor tanks (15-20ppt saltwater), for a three-week quarantine period, primarily as a precautionary action to kill any parasites. Until the growth trial, the fish were fed a typical commercial diet (BioMar A/S, Brande, Denmark).

2.2. Experimental diets

Dried *Sacharina latissima* sugar kelp from Integrated Multi-Trophic Aquaculture (IMTA) was used in the experimental feeds. The *S. latissima* seaweed had grown on longlines (robe on which the seaweed is deployed and harvested from) in close proximity to a commercial Norwegian Atlantic salmon farm, from October 2017 to May 2018. The dried *S. latissima* was analysed for iodine content before feed preparation to establish the appropriate dose of iodine and feed inclusion levels. Based on an iodine content in *S. latissima* of ~ 5g/kg dw, concentrations of iodine were added up to ~ 200 mg/kg (~ 4% kelp inclusion). Four iso-nitrogenous and iso-caloric diets were formulated and produced by SPAROS Lda, Olhão, Portugal (Table 1). Protein and starch sources of the reference diet were based on a mixture of fishmeal, soy protein concentrate, krill meal, faba beans, wheat gluten and wheat meal. Kelp meal was included in three of the four diets at 1%, 2% and 4% (w/w) respectively, replacing some of the wheat meal in the formulation. All diets were enriched with selenium by adding 0.02% selenium yeast. The oil coating on the pellets was a mixture of primarily rapeseed oil and, to a smaller extent, fish oil. For measurements of apparent absorption/ digestibility , yttrium oxide was added to all diets at the level of 0.01%.

2.3. Macronutrients and gross energy content analyses.

Feed samples were homogenised using a Speedy Pro homogenizer (Krups, Frankfurt am Main, Germany) and analysed for dry matter (DM) and ash (NMKL, 1991), crude protein (CP) (ISO, 2005, where protein = Kjeldahl-N x 6.25), crude fat (Bligh and Dyer, 1959) modified to fish feed and total phosphorus (ISO, 1998). Nitrogen free extract was calculated as DM-CP-fat-ash. The gross energy content was measured using an IKA calorimeter C7000 (Janke & Kunkel IKA Analysentechnik, Staufen, Germany) after drying the homogenized diet samples for 48 h at 60°C.

2.4. Element analyses

2.4.1. Total arsenic, cadmium, mercury, lead, selenium and yttrium

Total arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), selenium (Se) and yttrium (Y) were determined using inductively couple plasma mass spectrometry (ICP-MS) after acid pressure digestion, based on the principles of the European standard methods EN 13805:2014 and EN 15763:2009 as described by Rasmussen et al. (2017). In brief, subsamples (approximately 0.300 g dry weight) were digested in closed vessels in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria) with 4 mL HNO_3 (65% w/w) and 2 mL ultra-purified water (<18 MΩ cm; Milli-Q-Integral system, Merck, Germany). Digests were diluted to a volume of 20 mL with ultra-pure water. Prior to analysis, sample aliquots were further diluted with ultra-pure water and HCl added to obtain aqueous solutions of 3% HNO_3, 1% HCl (c/v). The elements were determined by ICP-MS (ICAPQ ICP-MS, Thermo Fischer Scientific, Bremen, Germany) using external linear calibration. Rhodium wad added as internal standard to correct any drift of the instrument. The limits of quantification were 0.0006 mg/kg for As, 0.0006 mg/kg for Cd, 0.0009 mg/kg for Hg, 0.012 mg/kg for Pb and 0.012 mg/kg for Se, respectively. Accuracy and precision were evaluated by analysis of certified reference materials (CRM). For DORM-4 (Fish protein, National Research Council Canada (NRCC), Ottawa, Ontario, Canada) the values obtained for most elements (As 6.67 ± 0.25 mg/kg, n = 6; Cd 0.320 ± 0.010 mg/kg, n = 6; Pb 0.451 ± 0.031 mg/kg, n =

106 6; Hg 0.422 ± 0.021 mg/kg , n = 5) were in agreement with the certified values (As 6.87 ± 0.44 mg/kg; Cd
107 0.299 ± 0.018 mg/kg; Pb 0.404 ± 0.062 mg/kg; Hg 0.412 ± 0.036 mg/kg), while for Se, the value obtained
108 (Se 4.95 ± 0.42 mg/kg, n = 6) was higher than the certified value (Se 3.45 ± 0.40 mg/kg). For BCR-668
109 (Mussel tissue, Joint Research Centre (JRC), Geel, Belgium) the values obtained (Y 56 ± 3 µg/kg, n =5)
110 were in agreement with the certified value (Y 59 ± 5 µg/kg).

111 *2.4.2. Iodine analysis*

112 Iodine (I) was determined by ICP-MS after alkaline extraction with dilute tetramethylammonium
113 hydroxide (TMAH) based on the principles of the European standard method EN 15111:2007 as
114 described by Camacho et al. (2018). In brief, subsamples of muscle tissue approximately 1.000 g; faeces
115 approximately 0.200 g; feed approximately 0.300 g were mixed thoroughly with 1 mL of TMAH (25%
116 w/w, 99.9999%) and 5 mL ultra-purified water (<18 MΩcm; Milli-Q-Integral system, Merck, Germany)
117 before being incubated at 90 ± 3 °C for 3 hours. After cooling, the extracts were diluted to 50 mL with
118 ultra-pure water. Before analysis, an internal standard (tellurium) was added and the extracts were further
119 diluted with 0.5% TMAH, depending on the expected iodine content. Iodine was determined by ICP-MS
120 (ICAPQ ICP-MS, Thermo Fischer Scientific, Bremen, Germany) using external linear calibration. The
121 limit of quantification for the method was 0.03 mg/kg. Accuracy and precision were evaluated by
122 analysis of a certified reference material (CRM). For BCR-422 (Cod muscle, JRC) the value obtained
123 (4.84 ± 0.26, n = 3) was in agreement with the certified value (4.95 ± 0.49 mg/kg).

124 *2.4.3. Inorganic arsenic analysis*

125 Inorganic arsenic was determined as sum of arsenite (AsIII) and arsenate (AsV) using anion exchange
126 HPLC (high performance liquid chromatography) coupled to ICP-MS, following acidic digestion based
127 on the principles of the European standard method EN 16802:2016 as described by Rasmussen et al.
128 (2017). In brief, subsamples (approximately 1.000 g) were extracted using 10 mL (aqueous 0.1 M HNO_3
129 in 3% H_2O_2) at 90 ± 3 °C for 1 hour and centrifuged (2,500×g, 10 °C) for 10 min. Hydrogen peroxide
130 ensured a quantitative oxidation of arsenite (AsIII) to arsenate (AsV) (Rasmussen et al., 2012). An aliquot

of the supernatant was transferred to a filter vial (Mini-UniPrep, PTFE Filter media with polypropylene housing; GE Healthcare, Little Chalfont, UK) prior to analysis. HPLC column outlet was connected to the ICP-MS nebuliser via a short length of PEEK tubing (0.13 mm id). Separation was obtained using a polymer-based strong anion exchange column (Dionex IonPac AS7, 10 µm, 2×250 mm) equipped with a guard column (Dionex IonPac AG7, 10 µm, 2×50 mm; 5 µl injection volume) by isocratic elution (0.15 mL/min, 15 min run time) using a HPLC system with a binary pump and autosampler (Agilent 1260 series, Agilent Technologies, Waldbronn, Germany). The mobile phase was prepared weekly by dissolving ammonium carbonate (50 mM) in 3% (v/v) methanol aqueous solution followed by adjustment of pH to 10.3 with 25% (v/v) aqueous ammonia and subsequently filtration through a 0.45 µm polyvinylidene difluoride filter (Millipore, Denmark) prior to use. Inorganic arsenic was determined by ICP-MS (Agilent 8800 ICP-QQQ-MS; Agilent Technologies, Santa Clara, CA, USA) using external linear calibration. The limit of quantification of the method was 10 µg/kg. Accuracy and precision were evaluated by analysis of a certified reference material (CRM). For ERM-BC211 (rice, JRC) the value obtained (110 µg/kg) was in agreement with the certified value (124 ± 11 µg/kg).

2.5. Trout trial

2.5.1. Trial facilities and conditions

Experimental protocols were prepared for all trials performed at DTU-Aqua, the North Sea Research Centre, and the Ministry of Food and Environment routinely inspects the facilities. No special permission from The Danish National Committee for the Protection of Animals was required for this trial as it did not include toxicants nor was it expected to cause pain or stress. Previous applications to the National Committee for the Protection of Animals used for scientific purposes, regarding fish trials performed by scientist/staff at the facility have been granted (e.g. Granby et al., 2018).

The facilities used for the trial consisted of 12 circular polyethylene rearing tanks (diameter 1m, ~600 L), supplied with aerated flow through fresh seawater. The water was, to some extent, temperature controlled, but due to the flow-through conditions and the high throughput, the temperature could only be increased from 7-8° to 11°C, which is less than optimal for rainbow trout (~15°C). However, the temperature remained stable throughout the trial. Each tank was equipped with circulation pumps placed at the wall of the tank, resulting in a circular current that drove uneaten pellets and faeces into a central bottom drain. The water current also seemed to lower aggressive interactions. An external standpipe on each of the 12 tanks was fitted with a whirl separator for collection of uneaten pellets and faeces. Oxygen saturation was continuously monitored in each tank using fixed oxygen probes connected to an oxygen delivery system. If oxygen saturation fell below 85%, pure oxygen was added to the tank through ceramic diffusers, and when saturation had increased to 90%, the supply was turned off. This resulted in a very stable oxygen saturation, also during feeding hours. Light condition were 14.5L: 9.5D cycles (light on 07:30-22:00 h).

2.5.2 Fish stocking and maintenance

A total of 561 fish were anaesthetized with benzocaine (200 mg/L, diluted as ~20 mL in 100L), individually weighed, and their lengths measured before being randomly distributed among the 12 tanks, i.e. 46-48 fish per tank. Average fish size was 196.1±25.6 g and 25.1±1.12 cm (mean±SD). Total biomass for each tank varied between 8.90 kg and 9.44 kg. The fish were fed experimental diets in triplicate for 12 weeks. Based on appetite observations, the fish were fed approximately 0.9% of expected biomass (average entire trial). The expected biomass between weighing was calculated using a feed conversion ratio of 0.8. Feeding was continuous by belt feeders for a period of about eight hours and uneaten pellets were recorded the following morning. All fish were individually weighed at four and eight weeks and at the end of trial (week 12), while lengths were measured only at the onset of the trial and at the end. Faeces were stripped after eight weeks in order to estimate apparent digestibility coefficients (ADCs) for nutrients (protein, lipid and phosphorus), and apparent absorption coefficients (AACs) for essential and potentially toxic elements were estimated the same way.

179 *2.5.3. Sampling*

180 At the onset of the trial, 48 fish were killed with an overdose benzocaine, and 12 were frozen whole for
181 later proximate composition analysis. The remaining fish (36) were allocated to nine pools, four fish in
182 each, for time t=0 measurements. Fillets were cut from both sides; the skin was left intact and only tissue
183 above the lateral line and in front of the anal fin was used. One fillet was bagged individually and the
184 other pooled, i.e. four fillets per pool. In addition, liver and kidney were removed, weighed and frozen in
185 pools. All tissues were frozen and stored at -20°C until further analyses. At the end of the trial, three
186 pools of 12 fish (only 10 in one of the tanks, see below) were sampled from each tank, resulting in nine
187 pools per diet for each tissue mentioned above. Muscle samples without skin and liver were also sampled
188 and flash frozen in liquid nitrogen for further evaluation of gene expression and analyses of lipids,
189 cholesterol and vitamin D (Ferreira et al. 2020, *in press*).

190 Pooled samples of whole fish were autoclaved for 3 h (120°C) and homogenised using a hand processor
191 before analyses of protein, lipid, dry matter and energy, as described for the diets. Sample pools of four
192 fillets with skin were homogenized for chemical analyses.

193 *2.5.4. Faeces*

194 Stripped faecal samples were frozen at -80°C, freeze dried (Christ Beta 2-16), and further dried overnight
195 at 60°C. The samples were powdered by hand and analysed for protein, lipid, dry matter and ash
196 phosphorus, as described for the diets in section *2.3*. A sub-sample was used for determination of yttrium,
197 and target elements (see section *2.4*).

198 *2.6. Performance calculations*

199 Apparent digestibility coefficient (ADC) respectively apparent absorption coefficient (AAC) were
200 calculated as:

201 ADC_X = 100-100 * ((%Y_{feed}/% Y_{faeces})*(%X_{feed}/% X_{faeces})), where Y=yttrium and X is the nutrient or
202 element of interest.

203 The specific growth rate (SGR, %/day) was calculated from biomass gain in the tanks, assuming that fish
204 grew in an exponential manner (Hopkins, 1992): SGR = 100*ln (W_t – W_0)/t, W_t where W_0 = biomass in a
205 tank at the start and end of the growth period, and t= number of feeding days. The biomass of fish dying
206 during the trial was added to the weight at the end of the growth trial, but all the fish that died, did so
207 during the final growth period (week 8 – 12). The feed conversion ratio (FCR) = feed intake/ biomass
208 gain (kg/kg). Protein efficiency ratio (PER) = biomass gain/protein intake (kg/kg). Protein retention (PR,
209 %), also called protein productive value was calculated from biomass (BM) and protein content of fish at
210 the end and start of each growth period and protein intake:

$$PR.(\%) = \frac{BM(end)x\ protein\ content\ (end) - BM\ (start)x\ protein\ content\ (start)}{protein\ intake} x\ 100$$

211 The biological value (BV) was calculated as:

$$BV = \frac{BM(end)x\ protein\ content\ (end) - BM\ (start)x\ protein\ content\ (start)}{protein\ intake\ x\ ADC\ (protein)} x\ 100$$

212 Lipid retention (LR) was calculated as:

$$LR.(\%) = \frac{BM(end)x\ lipid\ content\ (end) - BM\ (start)x\ lipid\ content\ (start)}{lipid\ intake} x\ 100$$

213 Energy retention (ER) was calculated from BM and energy content at the end and start of each growth
214 period and energy intake:

$$ER\ (\%) = \frac{BM(end)x\ energy\ content\ (end) - BM\ (start)x\ energy\ content\ (start)}{energy\ intake} x\ 100$$

215 Energy content of the four diets was measured by bomb calorimetry, while in fish it was estimated using
216 protein and lipid contents for the fish and energy values for protein and lipid (23.7 MJ/kg and 39.6
217 MJ/kg).

Hepato somatic index (HSI) also named liver somatic index was calculated as HSI = 100 x (liver weight/body weight).

2.7. Histological measurements of the intestine

The anterior intestine was collected after pyloric caecum (0.5cm fragments), from three fish per tank (nine fish per diet) for histological evaluation. Samples were fixed in 4% neutral-buffered formaldehyde and embedded in paraffin. Transversal sections of each sample were cut (3 µm-thick) in a semi-automated rotary microtome (Leica RM 2245, Leica Microsystems, Wetzlar, Germany). Slides were dewaxed and stained with specific Alcian Blue/PAS (pH 2.5). Micrographs of each section were taken with a 4x objective using an Olympus BX51 microscope and an Olympus SC50 camera (Olympus Corp, Tokyo, Japan). For each intestinal section, the following parameters were measured in two subsections using the imaging software Olympus cellSens Dimension Desktop: area and perimeter; total muscular layer thickness; fold length and width and goblet cell presence. The total muscular layer thickness was measured at eight points in each transverse section and the mean value considered. The same procedure was applied for thickness of the inner circular muscle layer. The thickness of the outer longitudinal muscle layer was calculated as the difference between the total muscular layer thickness and the thickness of the inner circular muscle layer. The eight longest folds in each section were measured (length and width) and goblet cells (mucus-producing cells) counted. Folds lengths were determined from the fold tip to the bottom, following the curves of the folds, whilst widths were determined at the bases of the folds.

2.8. Statistics

One-way ANOVA, followed by Holm-Sidak, significant differences ($p<0.05$) are denoted by different superscript letters. Where equal variance tests were not passed, a Kruskal-Wallis one-way Analysis of Variance on Ranks was performed. However, statistics of element concentrations in feeds and fillets were performed by one-way ANOVA analyses using the Excel Analysis ToolPak.

242

243 **3. Results and discussion**

244 *3.1. Diet composition*

245 Feed formulation, estimated concentrations of protein, fat, iodine, selenium and polyunsaturated fatty
246 acids (DHA+EPA) as well as analysed gross composition and gross energy contents appear from Table 1.
247 Measured gross composition values were close to those estimated, although protein decreased slightly
248 with increased kelp meal inclusion.

249 **Table** 1. Ingredients and composition of diets of control or 1%-, 2%- or 4%-*Saccharina latissima*.

	Trout diet			
Ingredients, %	**Con-trol**	**1% *S. latissima***	**2% *S. latissima***	**4% *S. latissima***
Fishmeal LT70	14.00	14.00	14.00	14.00
Krill meal	3.00	3.00	3.00	3.00
Soy protein concentrate	21.00	21.00	21.00	21.00
Wheat gluten	9.00	9.00	9.00	9.00
Wheat meal	6.34	5.34	4.34	2.34
Faba beans (low tannins)	10.00	10.00	10.00	10.00
Fish oil	8.96	8.96	8.96	8.96
Rapeseed oil	24.24	24.24	24.24	24.24
Vitamin and Mineral Premix	1.29	1.29	1.29	1.29
MAP (Monoammonium phosphate)	1.20	1.20	1.20	1.20
Se yeast	0.02	0.02	0.02	0.02
Astaxanthin	0.04	0.04	0.04	0.04

L-Lysine	0.30	0.30	0.30	0.30
L-Threonine	0.20	0.20	0.20	0.20
DL-Methionine	0.40	0.40	0.40	0.40
Yttrium oxide	0.01	0.01	0.01	0.01
Saccharina latissima		1.0	2.0	4.0
Total	100	100	100	100
As feed basis (estimated)				
Crude protein, % feed	39.3	39.3	39.3	39.3
Crude fat, % feed	35.0	35.0	35.0	35.0
Se, mg/kg	1.2	1.2	1.2	1.2
I, mg/kg	3.3	57	112	220
Proximate composition				
Crude protein, %	39.5	38.6	38.1	37.5
Crude fat, %	34.4	35.3	35.3	35.3
Dry matter, %	94.8	94.2	94,2	93.8
EHA+DHA, %	0.99	0.96	0.94	0.96
∑ PUFA, %	3.07	2.93	2.82	2.91
Ash, %	3.30	3.36	3.56	4.20
Phosphorus - P, %	0.70	0.67	0.67	0.67
NFE, %	17.50	17.00	17.30	16.80
Energy, kJ/g	24.8	24.8	24.8	24.6

250

251 *3.2. Trout trial*

252 *3.2.1. Growth performance*

253 Overall, the trout trial went well, except for an oxygen failure (one-day) in one tank (tank 11, diet-1%
254 kelp), at about eight weeks. This caused the death of six fish and some feed loss (~30% of offered feed)

255 the following day. Two days after the incident, feed intake was back to normal and the incident did not
256 affect the overall performance of the fish.

257 The mean weight in each diet group ranged from 194.5g to 198.5g at the onset of trial and the trout grew
258 to final mean weights ranging from 457.5g (diet-4%) to 484.3g for the control diet at the end week12 (84
259 feeding days) (Table 2). The overall appearance of the fish was very good for all diets, with condition
260 factors (CFs) increasing from 1.21 at the onset of trial to about 1.45 at the end of the trial (Fig. 1b), and no
261 significant differences were found among diets. Fish fed 4% *S. latissima* had significantly lower final
262 weights than those fed all other diets. Furthermore, performance data showed that, after eight weeks, fish
263 fed 4% *S. latissima* had a significantly lower SGR (mean 1.16 %/day) compared to diet-1% (mean 1.27
264 %/day) and diet-2% (mean 1.26%/day), but not significantly different from controls (mean 1.23%/day). In
265 the same period, FCR was significantly higher for diet-4% (0.78) compared to the other test diets (control
266 0.75; diet-1% 0.73 and diet-2% 0.73 respectively). Although fish fed diet-1% and diet-2% had slightly
267 better performance compared to control after eight weeks, i.e. higher SGR and lower FCR, no significant
268 differences were found among the groups after 12 weeks.

269 Protein efficiency ratio (PER) was significantly higher after eight weeks for diet-1% and diet-2%
270 compared to both control and diet-4%, which was probably related to the slightly lower protein contents
271 of these diets, increasing the differences observed in FCR. Again, after 12 weeks, no significant
272 differences were found. Protein retention ranged between 47.9% and 47.1% for all groups with fish fed
273 diet-4% showing the lowest retention (Fig.2), but there were no significant differences among the groups.
274 Lipid retention in the fish decreased with increased kelp meal inclusion and was significantly lower for
275 diet-4% (67.2%) compared to controls (75.1%). Similarly, energy retention decreased from 59.5% in
276 control fish to 55.3% in fish fed diet-4%, but no significant differences were found. The hepato somatic
277 indices (HSI) (Fig. 1a) were negatively correlated with kelp-meal inclusion, resulting in significantly
278 lower HSI for livers from fish fed diet-4% compared to the other diets. Fish store “excess” energy in the
279 liver, and HSI is used as indirect measure of energy status and is affected among others by both diet, fish

size, reproductive status as well as exposure to pollutants. In the present study a lower HSI of diet-4% thus indicates a poorer diet.

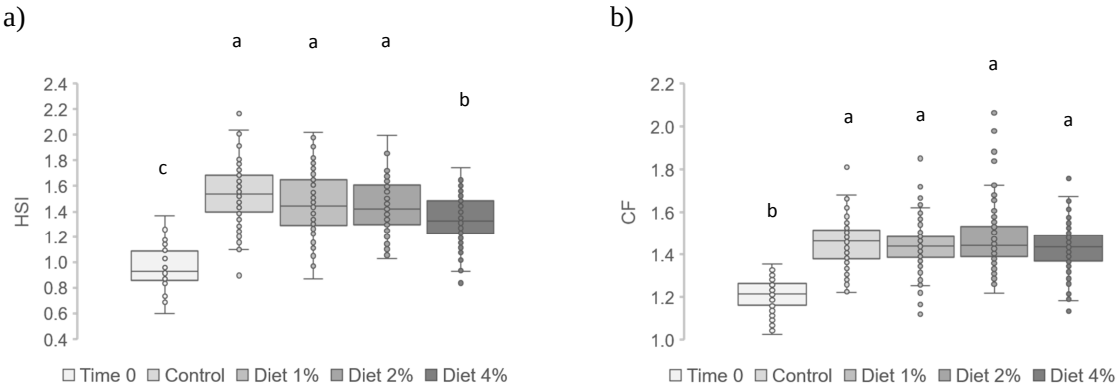

Fig. 1. a) Hepato somatic index (n=N=36, 108, 102, 108, 108); b) condition factor (n=N=140, 139, 134, 139, 136) in trout. Different superscript letters denote significant differences ($P < 0.05$).

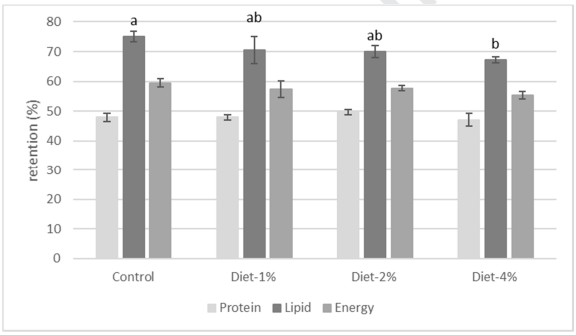

Fig. 2. Retention of protein, lipid and energy in trout fed control or diet with -1%,-2%,-4% *S. latissima*.for 12 weeks. Different superscript letters denote significant differences ($P < 0.05$).

292 **Table** 2. Growth performance and intestinal morphology of trout fed control-, 1%-, 2%- or 4%- *Saccharina*
293 *latissima* diets. Different superscript letters denote significant differences (P < 0.05).

Trout Diet	**Control**	**1% *S. latissima***	**2% *S. latissima***	**4% *S. latissima***
Growth performance				
Final fish weight (g),	484 ± 81[a]	477 ± 79[a]	481 ± 79[a]	458 ± 72[b]
Final fish length (cm)	32.1 ± 1.4 [a]	32.1 ± 1.5 [a]	32.0 ± 1.5 [a]	31.6 ± 1.4 [b]
SGR (%/day), 0-8 weeks	1.23 ± 0.034 [ab]	1.27 ± 0.020 [a]	1.26 ± 0.021 [a]	1.16 ± 0.043 [b]
SGR (%/day), 0-12 weeks	1.16 ± 0.009	1.13 ± 0.054	1.16 ± 0.012	1.10 ± 0.035
FCR (kg/kg), 0-8 weeks	0.751 ± 0.013 [a]	0.728 ± 0.010 [b]	0.733 ± 0.012 [b]	0.783 ± 0.010 [a]
FCR (kg/kg), 0-12 weeks	0.787 ± 0.023	0.818 ± 0.041	0.795 ± 0.010	0.836 ± 0.019
PER (kg/kg), 0-8 weeks	3.37±0.057 [b]	3.56±0.049 [a]	3.58±0.061 [a]	3.41±0.045 [b]
PER (kg/kg), 0-12 weeks	3.21±0.096	3.17±0.162	3.30±0.042	3.20±0.075
Histology				
Section area (mm^2)	12.9 ± 2.1	11.6 ± 2.0	13.5 ± 4.1	15.1 ± 2.8
Total muscular layer thickness (µm)	170 ± 22[a]	171 ± 23[a]	150 ± 29[ab]	130 ± 13[b]
Inner circular muscle layer thickness (µm)	112 ± 12[a]	111 ± 18[a]	95 ± 18[ab]	86 ± 8[b]
Outer longitudinal muscle layer thickness (µm)	58.3 ± 11.9[a]	59.2 ± 8.1[a]	55.1 ± 12.5[ab]	43.9 ± 7.11[b]
No. goblet cells/fold	85 ± 16	86 ± 15	96 ± 26	88 ± 15
No. goblet cells secreting acid mucins/fold	63 ± 14	56 ± 10	75 ± 28	68 ± 9
No. goblet cells secreting neutral mucins/fold	22 ± 11	29 ± 7	17 ± 8	23 ± 13
Width/fold (µm)	139 ± 13	136 ± 12	138.± 17	139 ± 17
Length/fold (µm)	762 ± 79	754 ± 75	802 ± 128	806 ± 108.

294 SGR: Specific growth rate: FCR: Feed conversion ratio; PER: Protein efficiency ratio; CF: Condition factor; HSI: Hepato somatic index

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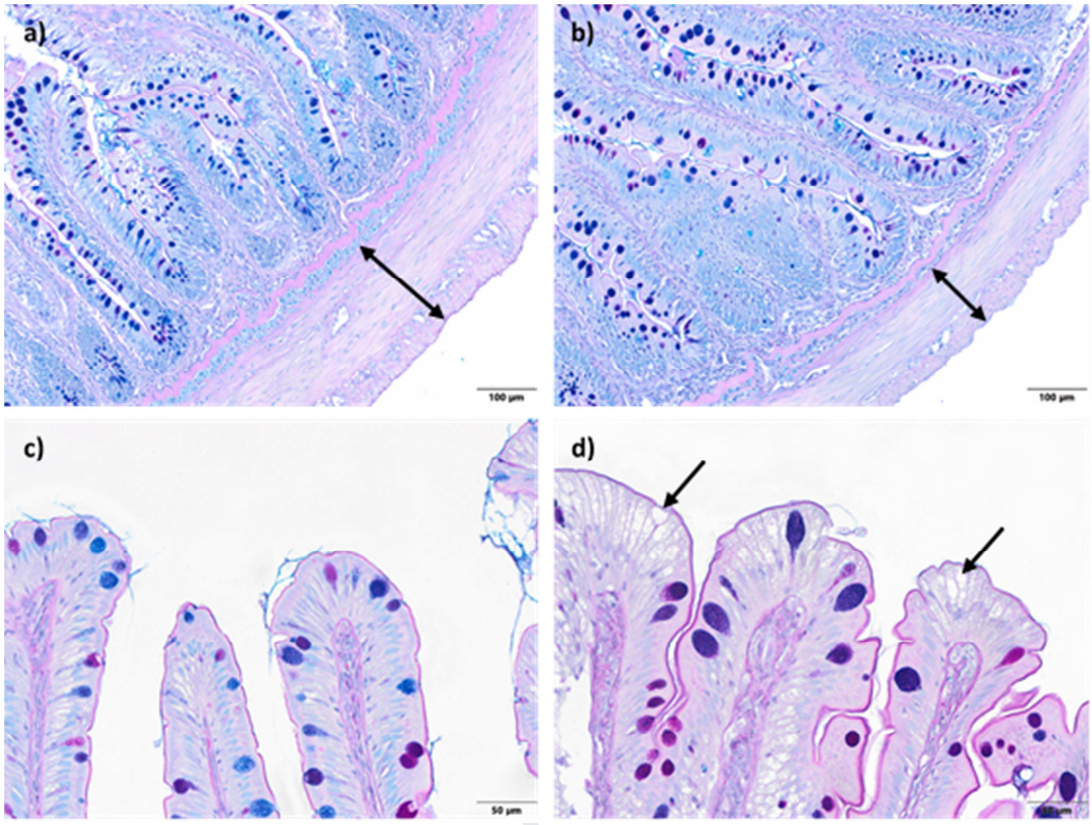

Fig. 3. Transversal paraffin embedded sections of anterior intestine (Alcina Blue-PAS staining), **a and c)** Intestine of fish fed control diet, **b and d)** Intestine of fish fed 4% *Saccharina latissima* diet. The 4-% diet shows lower thickness of muscular layer compared to the control. Arrows in d) are indicating several clear, variable sized, intracytoplasmatic vacuoles in the mostly apical enterocytes.

3.2.2. Intestinal histology and intestine thickness reduction

Intestinal villi heights, thicknesses and base widths of rainbow trout were evaluated at the end of the trial**.** Total muscular layer thicknesses in fish fed diet-4% showed a significant reduction compared to those fed with the control diet (Table 2, Fig. 3). Total section areas, villi widths and lengths, and number of goblet cells were not significantly affected by diet composition. Yet, in the mostly apical enterocytes of fish fed diet-4% variable sized intracytoplasmic vacuoles were observed (Fig. 3d). Growth impairment in fish fed seaweeds (10%) was previously associated with a significant reduction in intestine diameter in rainbow

trout (Araújo et al., 2016) and villi length in Nile Tilapia (Silva et al., 2015), which could adversely affect nutrient uptake. However, such results could not be confirmed in the present study. On the other hand, a decrease in tunica muscularis thicknesses, which could decrease intestine strength and motility, was noted with increasing *S. latissima* inclusion levels and was significant in fish fed diet-4% *S. latissima*. Previous studies reported increased growth performance and higher tunica muscularis thickness in trout fed fermentable fibre (Vitacel®, Yarahmadi et al., 2016). Regardless, the high fiber content of sugar kelp seems to compromise the fish intestinal histological structures, affecting nutrient digestibility.

3.4. Effects of kelp meal inclusion on apparent digestibility (ADC) or absorption efficiency (AAC) of nutrient, essential and potentially toxic elements

Apparent digestibility coefficient (ADC) is a measure of how much of the ingested feed ingredient is absorbed by the animal, i.e. the percentage of ingested nutrients/feed components not lost with faeces. It does not consider potential endogenous losses (e.g. digestive enzymes and mucoproteins excreted in faeces), hence the term "apparent". In theory ADC can be negative if the endogenous losses are larger than the absorption, e.g. if the animal actively excrete components via faeces. Here the term apparent digestibility coefficient (ADC) has been used for protein (N) and fat, as well as dry matter, nitrogen free extractives (NFE) and phosphorus, and apparent absorption coefficient (AAC) for essential and toxic elements, despite being calculated in the same way (Storebakken et al., 2000).

In the present study, inclusion of sugar kelp meal caused a small (~2.5%) but significant decrease in ADC of protein (N) from 90.2±0.3% in control diet to ~88±1% (n=9) for all fortified diets, i.e. digestibility did not decrease further with increased inclusion of kelp meal (Fig. 4). In contrast, ADC of fat ranged from 91.2±2.1% for diet-4% to 92.2±1.8% for diet-1%, showing no significant differences among groups. ADC of NFE were clearly affected by kelp meal inclusion, as diets-1%, -2% and -4% showed a significant (20%) lower ADC (decreasing from 51.7±0.9% to 40.6±2.2%) compared to the control diet.

However, the decrease did not correlate with the kelp inclusion levels. ADC of phosphorus increased slightly from 74.2±0.9% (control) to 75.6±0.7% (diet-4%), but these differences were not significant.

AAC for iodine, selenium and arsenic were strongly affected by sugar kelp inclusion, particularly between control and diet-1%, and to a lesser extent by further increase in sugar kelp content (Fig. 5). Iodine AAC was significantly affected by kelp inclusion, with a large relative increase ~30% from 62±4% in controls to 80±2% in fish fed diet-1%. It increased up to 83±0% for diet-4% but the differences were not significant among diets-1%, -2% and -4%.

In contrast, selenium, was *negatively* affected by kelp meal inclusion, as fish fed diets-1%, -2% and -4% had significantly lower AAC values (ranging between 58±4% and 66±3%) compared to those fed the control diet (73±4%), but there were no significant differences among the three diets with sugar kelp. Arsenic, which feed concentrations increased with kelp meal inclusion (Fig. 7d) decreased significantly in AAC from about 81±1% for the control diet to 61±1% for diet-4%. AAC of arsenic appeared to be somewhat correlated with kelp inclusion level, decreasing from 67±5% for diet-1% to 63±4% for diet-2%, but none of these differences were significant. Two elements (iron and cadmium) showed more AAC variability, however no significant differences were found among diets and they were neither correlated with levels of sugar kelp inclusion. AAC ranged between 32±7% - 40±2% for cadmium and were ≤5% for iron.

Studies on iodine AAC from kelp are scarce. Using an *in vitro* method with the human Caco-2 cell line Domínguez-González et al. (2017) found iodine bioaccessibilities of 49-82%, i.e. availability for absorption after seaweed gastrointestinal digestion. Aquaron et al. (2002) found iodine bioavailabilities from seaweeds to humans, for *Laminaria hyperborea* 62-90% and for *Gracilaria verrucosa* 85%-100%. The present study on trout found iodine AAC from *S. latissima* of 80-83% for iodine concentration 63-239 mg/kg.

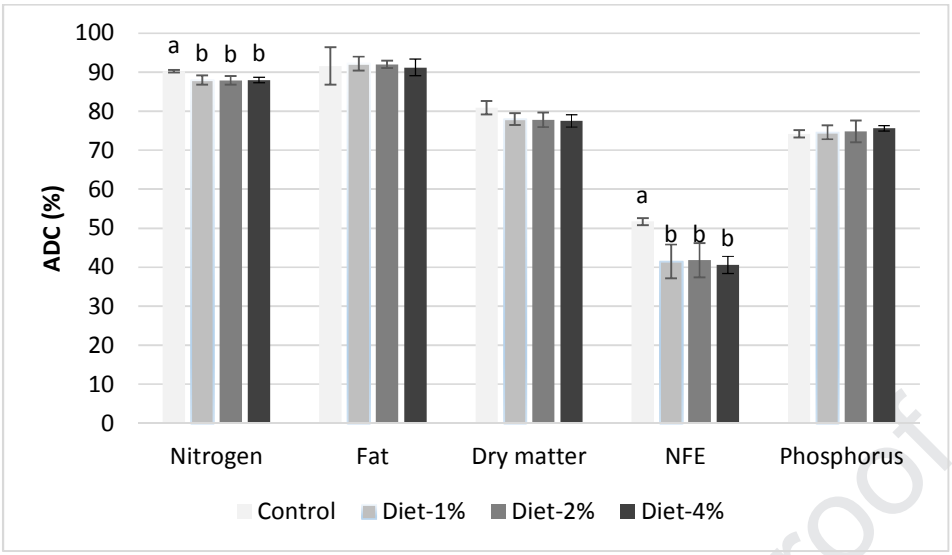

Fig. 4. Apparent digestibility coefficient (ADC)(%) of nutrients [nitrogen, fat, dry matter, NFE and phosphorous in trout fed control or 1%-, 2%-, 4%- *Saccharina latissima* diets (n=3) for 12 weeks. Different superscript letters denote significant differences ($P < 0.05$).

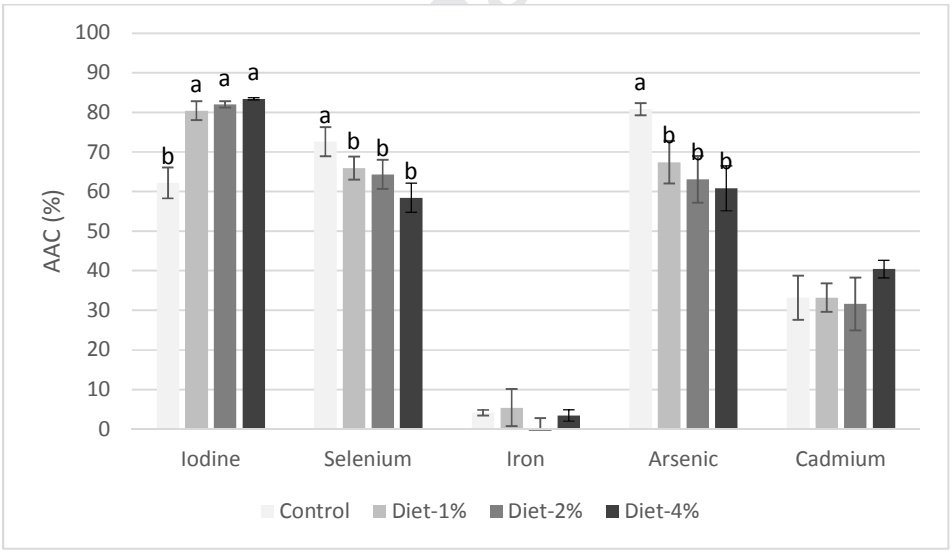

Fig. 5. Apparent absorption coefficient (AAC)(%) of essential and potentially toxic elements (iodine, selenium, iron, arsenic, cadmium) in trout fed control or 1%-, 2%-, 4%- *Saccharina latissima* diets (n=3) for 12 weeks. Different superscript letters denote significant differences ($P < 0.05$).

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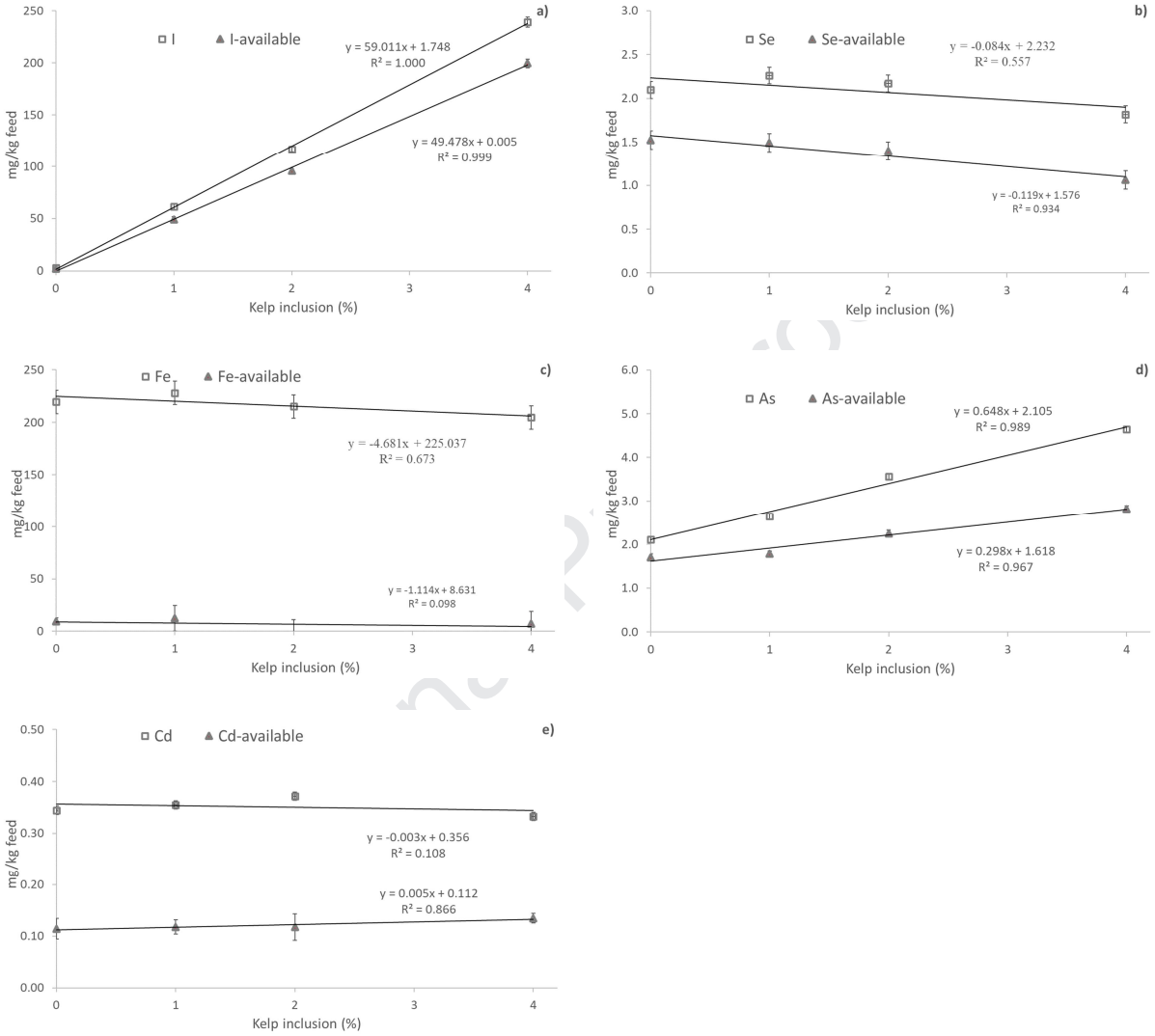
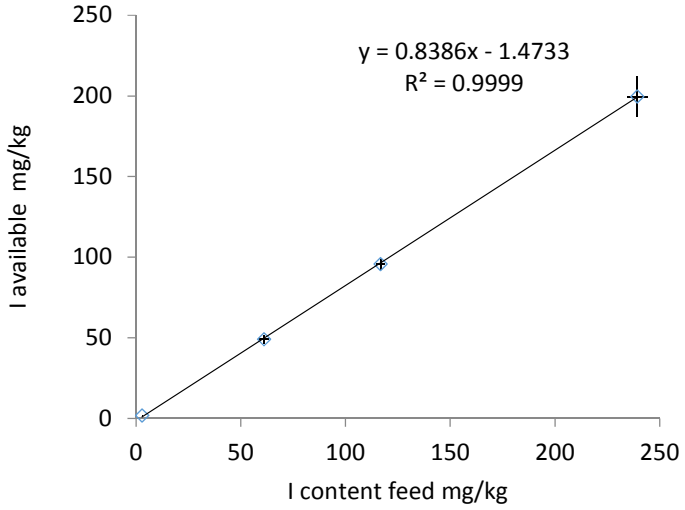
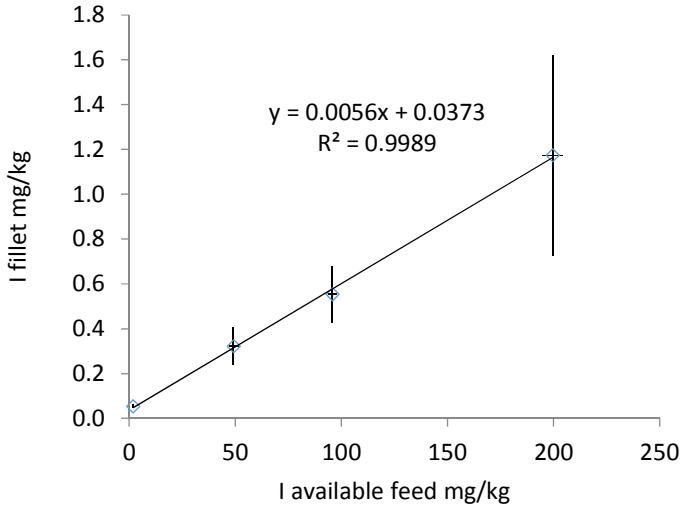


369 **Fig.** 6. Element concentrations in feed (total and available) versus % *Saccharina latissima* inclusion (element

370 available = total element concentration x AAC) a) iodine, b) selenium, c) iron, d) arsenic, e) cadmium.



a)



b)

371 **Fig.** 7. Iodine content a) available (absorbed) from feed *vs* total concentration in feed, b) in trout fillet *vs* available

372 (absorbed) from feed.

373 **Table** 3. Concentrations of essential and potentially toxic elements in control diet and three fortified diets of 1%; 2%
374 and 4% sugar kelp and in the trout fillets of fish fed the respective diets for 12 weeks. Different superscript letters
375 denote significant differences ($P < 0.05$).

Trout Diet	**Control**	**1% *S. latissima***	**2% *S. latissima***	**4% *S. latissima***
I feed mg/kg	3.6 ± 0.1	62.8 ± 2.3	117 ± 2	239 ± 5
I fillet mg/kg	0.055 ± 0.008 [d]	0.323 ± 0.08 [c]	0.55 ± 0.12 [b]	1.17 ± 0.45 [a]
Se feed mg/kg	2.10 ± 0.03	2.26	2.17	1.82
Se fillet mg/kg	0.21 ± 0.02	0.24 ± 0.03	0.22 ± 0.09	0.16 ± 0.06
Fe feed mg/kg	220 ± 11	228	216	205
Fe fillet mg/kg	2.80 ± 0.08 [b]	2.81 ± 0.02 [b]	2.95 ± 0.14 [b]	3.29 ± 0.14 [a]
As feed mg/kg	2.10 ± 0.06	2.64	3.56	4.64
As fillet mg/kg	0.77 ± 0.03	0.79 ± 0.05	0.81 ± 0.02	0.78 ± 0.02
iAs feed µg/kg	103 ± 1	n.a.	n.a.	109 ± 3
iAs fillet µg/kg	<3[1)]	<3[1)]	<3[1)]	<3[1)]
Cd feed mg/kg	0.345 ± 0.014	0.355	0.371	0.333
Cd fillet mg/kg	<0.0002[1)]	0.0003 ± 0.0003	<0.0002[1)]	0.0004 ± 0.0001
Pb feed mg/kg	0.069 ± 0.011	0.055	0.049	0.062
Pb fillet mg/kg	0.005 ± 0.001	<0.004[1)]	0.005 ± 0.007	0.018 ± 0.003
Hg feed mg/kg	0.057 ± 0.002	0.062	0.061	0.059
Hg fillet mg/kg	0.032 ± 0.003	0.031 ± 0.000	0.032 ± 0.001	0.031 ± 0.001

[1)] <LOD

376

377 *3.5. Essential and potentially toxic elements concentration in feed and trout fillets*

378 *3.5.1. Iodine*

379 Iodine concentrations in trout fillets were proportional to concentration absorbed by the fish from the feed
380 (Fig. 7b), i.e. inclusion of 1, 2, and 4% *S. latissima*, in the feed was highly correlated with iodine

concentrations in the trout fillets after 12 weeks (R^2=1.00). Nonetheless, iodine concentrations in biofortified fillets were only ~0.5% (0.51%; 0.47%; 0.49%) of iodine concentrations in the feed, despite up to 83% of iodine in feed was absorbed by the trout (Fig. 5). This is because the major proportion of available iodine was distributed to and retained by the thyroid gland where iodine is essential for thyroid hormones biosynthesis (Berson, 1956). Mean iodine concentration in fish fillets increased from 0.05 mg/kg at of the beginning of the trial, to 0.55 mg/kg for diet-2% after 12 weeks.

Iodine biofortification using 0.8 % dw of the brown macroalgae *L. digitata* (~4 g I/kg dw) in the feed of freshwater char (*Salvelinus* sp.), was assessed by Schmid et al. (2003). Final iodine concentration was 0.54 mg/kg ww in fillets with skin after nine months, which is comparable to the present findings, i.e. 0.55 mg/kg ww in trout fillets with skin (10-fold of control diet), using 2% dietary inclusion of *S. latissima* for three months. Schmid et al. (2003) also found ~5 times higher iodine concentrations in char skins compared to fillets without skin.

Valente et al. (2015) doubled iodine contents, from 0.11 to 0.22 mg/kg, in rainbow trout fillets after feeding fish for 91 days with 5% of red algae *Gracilaria vermiculofylla* (i.e. 105 mg I/kg dw). Growth of fish fed the test diet was not different from the control group, but increasing algae inclusion from 5% to 10% seriously impaired growth performance. Another rainbow trout trial (freshwater, 91 days) performed by Ribeiro et al. (2017), showed that addition of ~0.4% *L. digitata* (~20 mg I/kg feed) significantly increased iodine concentrations by six-fold, from 0.02 to 0.12 mg/kg fillet. The addition of *L. digitata* alone, or in combination with selenium, was associated with higher whole-body weights compared to controls. Likewise, Ribeiro et al. (2015) found that feeding seabream with 10% *L. digitata* (~428 mg I/kg dw feed) for 118 days resulted in a significant (6.5-fold) increase (to 0.84 mg/kg fillet) of fillet iodine content, over levels found in controls. Seabream seemed less sensitive to high dietary iodine and kelp inclusion, as feed intakes and efficiencies, growth rates, and nutrient utilization were not affected.

3.5.2. Selenium and iron

Selenium was added as selenised yeast in the same amounts to all diets, and fillet concentrations were not significantly different. Selenium had a ratio of ~10% in fillet relative to feed and iron had a ratio of ~1.5%. The iron contents in the trout fillet were significantly higher for the 4% diet compared to the control diet, but with no statistical differences found among the other diet combinations. The iron concentrations in fillet increased from 2.8 mg/kg in fish fed control diet to 3.3 mg/kg in fish fed 4%-diet. The iron feed concentrations did not increase with sugar kelp inclusion, and nor did AAC.

3.5.3. Arsenic, cadmium, lead and mercury

Arsenic concentrations in the test diets increased with inclusion of seaweed, meaning that *S. latissima* is a source of As, which is in accordance with data from a Norwegian survey of 21 species of seaweed showing that brown algae, including *S. latissima*, contain elevated levels of As (Biancarosa et al., 2018). Although As concentrations in the diets increased, AAC, i.e. the relative availability decreased with addition of seaweed, which suggests that As species present in seaweed are less bioavailable than those in fishmeal and fish oil, which are the main sources of arsenic in the test diets. The major form of As in seaweed are arseno-sugars, i.e. arsenic containing ribosides (Francesconi and Edmonds, 1998; Feldmann and Krupp, 2011). As, accumulated in trout fillets fed with the experimental diets, revealed no differences between the experimental groups, indicating that the source of the accumulated As are fish meal and fish oil. Fish meal may contain inorganic arsenic, which is carcinogenic (EFSA, 2009). The level of inorganic arsenic was low in the diets (5.0% of total As in the control diet, and 2.4% of total As in the 4%-diet) and inorganic arsenic was not found (< LOQ) in trout fillets fed with the experimental diets. The level of inorganic arsenic was also low (103 ± 4 µg/kg, n = 2) in the kelp included in the diets. In comparison, a different sample of *S. latissima* contained 250 µg/kg inorganic arsenic (Biancarosa et al., 2018). Cd concentration in the test diets did not increase with inclusion of seaweed, i.e. *S. latissima* was not a source of Cd, which is not atypical for seaweed (Biancarosa *et al.*, 2018). Cadmium did not accumulate in the trout fillets, despite the test diets contained some Cd (mean 0.3 mg/kg) and the Cd availability (AAC) was 30-40%, depending on the diet composition. Available Cd is likely to have accumulated in the intestines,

430 livers and kidneys of trout. Studies with Atlantic salmon (*Salmo salar*) have shown that Cd does not
431 accumulate in the fillet, but rather in organs (i.e. intestine, liver and kidney, Berntssen et al., 2001).
432 Concentations of Pb and Hg were low in all diets and did not increase with the inclusion of seaweed.
433 Seaweed, including *S. latissima*, typically contain low concentrations of Pb and Hg (Biancarosa et al.,
434 2018), and is not a source of either elements. Pb also did not accumulate in fillet from fish fed the test
435 diets, while Hg accumulated in the fillet, although at low levels.

436 When comparing the ratio of concentrations in fillets to those in the feeds, there were major differences
437 (Table 3) reflecting toxicokinetic characteristics of the different potentially toxic elements. Mercury
438 accumulated in muscle tissue (e.g. high ratio of ~50%), while Cd mainly occurred in liver and kidney
439 (ratio <0.1% to fillet). Fillets had low concentrations and high uncertainty for Cd and Pb and, in some
440 cases, values were below the limits of detection. It was interesting to note the decreased ratio of total
441 arsenic in fillets relative to feed with increasing seaweed inclusion (Table 3), which is probably due to the
442 corresponding decrease in AAC (Fig. 6d).

443

444 *3.6. Prospects for iodine biofortification*

445 It is important to have an appropriate level of iodine for function of the thyroid grand as the dose adverse
446 effect is U-shaped (Laurberg et al 2010), i.e. both deficiency and excess can affect thyroid hormone
447 regulations in humans, as well as in fish. In Europe, EFSA has set the tolerable upper safe limit (UL) to
448 600 µg/day for adults (EFSA, 2014).

449 Regarding minimum concentrations required for *Oncorhynchus* species, they are reported to be 1-1.1 mg
450 I/kg diet (NRC, 2011). Appropriate and/or upper addition levels of I in the form of a dietary supplements
451 have been studied in other trials. Schuhmacher et al. ('personal communication' in EFSA, 2005) found no
452 effects on performance and histomorphology of the thyroid gland in rainbow trout (*Oncorhynchus mykiss*)
453 and common carp (*Cyprinus carpio*) supplemented with iodine (0-64 mg/kg feed), even at the highest

dietary concentration (64 mg iodine/kg). Nor were columnar epithelial cells, as markers of epithelial hypertrophy, or proliferation of epithelial cells indicating hyperplasia observed. Julshamn et al. (2006) fed Atlantic salmon (*Salmo salar*) diets containing different amounts of I (10-86 mg/kg) for five months, but did not observe any effects on fish weights and lengths or plasma hormone (thyroxin (T4) and triiodo-thyronine (T3)) concentrations. Their results were in the same tolerable range as in the present study, where no adverse effects were observed with the inclusion of *S. latissima* in feeds corresponding to iodine concentrations of 63-117 mg/kg, while 239 mg/kg (4%-diet) affected trout growth performance parameters and significant histomorphological changes could be observed in the intestines. Furthermore, in terms of physiological effects in trout (Ferreira et al. 2020, *in press*) reported limited effects of kelp inclusion on iodine or growth-related genes, but a downregulation of genes associated with lipid metabolism (*FAS* expression) and oxidative stress (*GPx1b2* expression*)* in fish fed with the diet containing 4% kelp.

Iodine from consumption of farmed rainbow trout fed on a diet containing sugar kelp could contribute to recommended daily intakes (RDI) (150 µg per day, WHO, 2004). Based on 2% dw sugar kelp (117 mg/kg I) in the feed, iodine concentration in fillets was ~0.55 mg/kg. A portion of 160 g would correspond to an iodine intake of ~90 µg/day, which is about 60% of the WHO RDI of 150 µg and ~15% of the upper safe limit of 600 µg/day. Consuming 160 g of 2%-diet *S. latissima* fortified fillet would also correspond to half RDI for Vitamin D (15.0 µg/day).

Considering seafood (fish, shellfish and seaweed) has been a major source of I, using I-rich seaweed as ingredient in aquafeed to tailor fish products would seem a sensible approach. Low inclusion (< 4%) of *S. latissima* as natural ingredient in farmed fish feed might contribute to improved fish and human health by increasing I intakes. Other bioactive components of seaweed might further promote sustainable biofortification for health benefits in fish and humans.

4. Conclusions

479 The present rainbow trout trial showed a beneficial I biofortification with no detrimental effects at 2% *S.*
480 *latissima* in fish feed. Iodine fortification of feed up to 4% *S. latissima* (~239 mg/kg I in feed), was
481 associated with a 0.5% proportional I concentration in trout fillets (R^2= 1.00). Mean I concentrations in
482 fish fillets increased from 0.05 mg/kg at of the beginning of the trial to 0.55 mg/kg for diet-2% after 12
483 weeks. Consumption of a 160 g portion of such a biofortified trout fillet from fish fed a 2% *S. latissima*
484 diet would ensure ~ 60% of iodine RDI.

485 AACs of iodine were >80% for diets with *S. latissima*, while for Se and As, AACs *negatively* affected by
486 *S. latissima* inclusion. Although As from the kelp increased feed concentrations, the availability (AAC)
487 decreased with addition of seaweed, which suggests that As species present in seaweed are less
488 bioavailable than those in fishmeal and fish oil.

489 Regarding the potentially toxic elements inorganic As occurred in the feed, but could not be detected in
490 the fillets whilst Cd, Hg, Pb occurred in very low concentrations in fillets.

491 Fish fed 4% S. *latissima* had significantly lower final size, lower HSI and reduced tunica muscularis
492 thicknesses, which could decrease intestine strength and motility. In comparison 1%- and 2%-diets did
493 not affect fish growth performance. Inclusion of kelp also resulted in a significant decrease in protein (N)
494 ADC, without a major impact on FCR, after the 12 weeks biofortification.

495 Overall, this study clearly demonstrates that inclusion of up to 2% of *S. latissimi*, as an ingredient in
496 aquaculture feeds, can proportionally transfer I to fish, at concentrations that would improve human
497 iodine intake and status, as well as other bioactive substances (e.g. ω3-polyunsaturated fatty acids and
498 vitamin D), thus contributing to the health benefits of seafood for European consumers.

499

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Highlights

- Fortification of trout with no impact at 2% *S. latissima* (117 mg iodine/kg) in feed
- Iodine up to 239 mg/kg in feed proportional to trout fillet transfer (0.5%)
- Apparent absorption coefficients of arsenic, cadmium, iodine and selenium
- Toxic elements in feed, occurred in low concentrations in fillets (As, Cd, Hg, Pb)
- Consuming of a 160 g fillet contributes ~60% of recommended daily iodine intake

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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