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A commercial macroalgae extract in a plant-protein rich diet diminished saturated fatty acids of *Oncorhynchus mykiss* walbaum fillets

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ABSTRACT

Seaweeds are considered novel feed ingredients, nutraceutical compounds and source of pigments and proteins. They appear to possess bioactive properties, such as hypolipidemic, antioxidant and immune-stimulative actions; furthermore, their proteins are considered just as nutritious as terrestrial vegetables. In the present work, rainbow trout (*Oncorhynchus mykiss* Walbaum) was fed for 95 days with three diets: a fishmeal-based positive control diet (C+), a vegetable protein-based negative control diet (C−) and a diet similar to C − where 5% of soybean concentrate was replaced by a commercial blend of seaweeds (T). The monitored parameters were fish performance, physical and marketable characteristics, fillet oxidative status and fatty acid content. The estimated indices of enzyme activities involved in lipid metabolism were calculated. Fish performances were reduced in C − and T fish; C − and T fillet fatty acid (FA) profiles globally showed the same pattern and were distinct from C+, i.e. with a lower content of polyunsaturated FAs and a higher content of monounsaturated and polyunsaturated n6 FAs; contrarywise, saturated FAs were significantly lower in T group in comparison to the other two groups ($p < .01$). The estimated indices of enzyme activity highlighted differences between dietary groups; desaturase activities of C18 and n3 FAs were higher in T in comparison to C− ($p < .001$). A slight impoverishment of antioxidant activity was found in T compared to C + group. Dietary seaweeds seemed to be capable of influencing fillet FA composition as well as the activity of enzymes related to lipid metabolism. Further studies in this regard are encouraged.

HIGHLIGHTS

- Seaweeds are novel feed ingredients with nutraceutical properties
- Saturated fatty acids of rainbow trout fillets were lowered by seaweed inclusion in the diet
- Estimated desaturase activities of C18 and n3 fatty acids were higher in seaweed-fed trout

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Introduction

The intensification and expansion of farmed fish production necessitate larger volumes of protein for feed formulation and in the last decades vegetable proteins have been the chief substitute of fishmeal. Aquaculture industry takes particular consideration for this aspect as vegetable substitutes rarely have an optimal fatty acid (FA) profile and often contain anti-nutritive factors (Oliva-Teles et al. 2015). In addition, fish products are well-known for their high polyunsaturated fatty acid (PUFA) content and feed FA profile could negatively affect the fish characteristics. As counter-action, supplements and functional ingredients could be added to feeds in order to carry nutraceutical molecules and have been studied to leap over the hurdles carried by vegetable sources.

Among functional ingredients, seaweeds are promising. Base of the aquatic food chain (Norambuena et al. 2015) and thus eco-friendly, with a potentially high availability, their production lined up at 30.1 million tonnes (wet weight) in 2016 (FAO 2018). For a long time, macroalgae have been used for ecosystem

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Supplemental data for this article can be accessed here.

services, in polyculture systems and in water remediation; more recently, research has pointed in the direction of using them as fodder supplements and alternative protein source for farmed fish (Hasan and Chakrabarti 2009). Indeed, macroalgae enclose in themselves an enormous potential, being a source of polysaccharides, lipids, proteins, minerals, vitamins, various pigments and polyphenols (Holdt and Kraan 2011). The nutritional value of algal proteins is no less than that of terrestrial vegetables. Macroalgae typically contain a low lipid amount, but the predominant FAs are eicosapentaenoic acid (EPA) and other n3 and n6 PUFA, in a balanced mix (Holdt and Kraan 2011). In addition, algae contain compounds with bioactive features, such as immune-stimulative properties, antibacterial and antiviral actions, hypocholesterolaemic and hypolipidemic effects and antioxidant activity (Holdt and Kraan 2011; Samarakoon and Jeon 2012).

On the downside the chemical composition of seaweeds is subjected to high variation depending on species, environment, time and geographic space, moreover, caution must be kept as regards too high concentrations of minerals or the presence of heavy metals, therefore generalisations about their characteristics should not be made (Hasan and Chakrabarti 2009; Holdt and Kraan 2011). There are 33,260 species named in AlgaeBase (Guiry and Guiry 2017), usually grouped for commercial purposes in green, red, brown, yellow-green algae, roughly corresponding to Chlorophyta, Rhodophyta, Phaeophyceae and Xanthophyceae taxonomic groups, respectively, but many other groups exist (Hasan and Chakrabarti 2009).

As a consequence of such a variety, different results can be found in the literature when they are used as feed ingredients. For instance, some research studies did not find any effect of a low-level inclusion of dietary seaweed on growth performance and protein efficiency ratio of several fish species (Hasan and Chakrabarti 2009; Soler-Vila et al. 2009; Güroy et al. 2013; Peixoto et al. 2016). However, early studies have shown that low levels (2.5–10% of the diet) of algae in fish diets have positive effects on many characteristics of fish, such as growth, disease resistance and end-product quality, while other studies detected negative results (Norambuena et al. 2015).

The present work aimed to assess the suitability of replacing 5% of soybean concentrate with a commercial blend of seaweeds in a feed for rainbow trout (*Oncorhynchus mykiss* Walbaum) with a high content of vegetable sources, estimating indices of the lipid metabolism and monitoring the effects on growth performance and fillet marketable, physical and chemical composition.

Material and methods

Experimental diets and growth trial

The present trial was performed according to the European Directive 2010/63/EU (Directive 2010/63/EU 2010) of the European Parliament and of the Council of European Union on the protection of animals used for scientific purposes. The fish farming and sampling phases were performed at the Edmund Mach Foundation experimental fish plant, located in San Michele all'Adige, Trento, Italy (Ministerial Clearance n. 120/2008-A).

A number of 900 female rainbow trout (183.3 ± 29.6 g, mean ± SD) was randomly divided into three dietary groups and allocated to a total of nine fibreglass tanks (three tanks per dietary group). The water volume in each tank was set to 3.5 m^3, in a flow-through system (mean flow 4.85 L/sec; mean temperature 12.1 °C). Water temperature and dissolved oxygen in the water outlet was measured weekly, while the administered feed and the mortality was recorded daily for each tank. Fish were fed manually 6 days a week. Feeding ratios were adjusted weekly as the fish grew (total biomass was assessed every third week). Feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) were evaluated at the end of trial.

After 2 weeks of acclimatisation, fish were fed for 95 days (65 meals) with three different isonitrogenous, isolipidic and isoenergetic diets (Table 1). A positive control (C+) was formulated containing 53% of fish meal and 15.1% of fish oil (w/w); a negative control (C−) was represented by a diet where marine sources were strongly substituted by protein and oil from vegetable sources; the third diet (T) was formulated basing on C − with a 5% replacement of the soybean meal with a commercial blend of seaweeds. This level of dietary seaweed inclusion was chosen basing on the suggestion of the blend producer. The producer only disclosed that the blend is composed of 11 selected species of brown, green and red seaweed, excluding *Ascophyllum*, *Fucus* and kelp. The colour and the FA profile of the three experimental diets were analysed with the methodology hereunder described.

Sampling and marketable traits

Three sampling times were set. Six fish in total were sampled after the acclimatisation (T0), six fish per

Table 1. Formulation, proximate composition and FA groups content of the extruded experimental diets.

	C+	C−	T
Ingredients (% as fed)			
Fishmeal 999	53.2	6.2	6.2
CPSP 90 IDR	6.0	6.2	6.2
Wheat gluten	8.5	10.3	10.3
Corn gluten	0.0	12.4	12.4
Soy protein concentrate	8.5	10.3	10.3
Commercial blend of seaweeds	0.0	0.0	5.2
Soy meal extract (48%)	0.0	25.8	20.6
Fish oil	15.1	8.2	8.2
Rapeseed oil	6.0	11.9	11.9
Idropalm	2.4	3.6	3.6
Vitamin premix	0.1	0.1	0.1
Oligomineral premix	0.1	0.1	0.1
Dicalcium phosphate	0.0	2.6	2.6
DL-Methionine	0.0	0.5	0.5
Lysine-HCL	0.0	1.5	1.5
Betaine	0.0	0.3	0.3
Total	100.0	100.0	100.0
Fish protein (% of crude protein)	70	10	10
Fat coming from fish (% of crude fat)	70	35	35
Chemical composition			
Crude protein (% on a.i. basis)	41.2	41.1	41.0
Crude fat (% on a.i. basis)	24.4	24.2	24.3
Fatty acid groups (g/100 g total FAME)			
ΣSFA	22.85	27.43	29.94
ΣMUFA	42.89	40.19	40.66
ΣPUFAn6	14.95	19.92	18.11
ΣPUFAn3	18.47	11.93	10.77
PUFAn3/PUFAn6	1.24	0.60	0.60

FA: fatty acid; C+: positive control; C−: negative control; T: third diet; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

dietary group were sampled after six weeks (T1) from the beginning of the experimental feeding and at the end of the trial (T2), i.e. when rainbow trout reached the marketable weight and size. Prior to each sampling, the fish were starved for 1 day, euthanised by a sharp blow to the head, then frozen at −80 °C and shipped to the laboratory where fish were stored at the same temperature until the analyses. Prior to analysing, the fish were thawed overnight at +1 °C. Firstly, the colour of the skin was measured on triplicate positions (cranial, medial and caudal) on both skin sides with a CHROMA METRE CR-200 (Konica Minolta, Singapore Japan) following the CIELab system (CIE 1976) and recording L* (lightness), a* (redness index) and b* (yellowness index) parameters. Then, the fish were individually weighed, measured, and dissected to calculate the following parameters:

$$\text{Condition factor, CF } (\%) = \frac{BW}{TL^3} \times 100$$

$$\text{Fillet yield, FY } (\%) = \frac{FW}{BW} \times 100$$

where BW is the body weight (g), TL is total length (cm), FW is the weight of fillets with skin (g).

The hepatosomatic (HSI) and visceral somatic (VSI) indices were also calculated.

Colour values of both right and left fillets were recorded and ΔE between samples was calculated according to the following formula:

$$\Delta E_{(\beta-\alpha)} = \left[\left(L^*_\beta - L^*_\alpha\right)^2 + \left(a^*_\beta - a^*_\alpha\right)^2 + \left(b^*_\beta - b^*_\alpha\right)^2 \right]^{0.5}$$

where α and β represent alternatively the mean colour values of C+, C − and T.

Finally, C+, C − and T right fillets of trout fed the diets for 6 and 12 weeks (T1 and T2, respectively) were analysed as fresh for physical and chemical characteristics.

Fillet physical characteristics

The values of pH, maximum shear force and water holding capacity (WHC) parameters were considered. The pH value was measured on triplicate fillet positions (cranial, medial and caudal) by a pH-metre SevenGo SG2™ (Mettler-Toledo, Schwerzenbach, Switzerland). Texture was assessed as the maximum shear force value obtained after a 50% Warner-Bratzler shear test; a Zwick Roell® 109 texturometer (Zwick Roell, Ulm, Germany), equipped with a 1 kN load cell and a straight blade (width of 7 cm), was used to perform the test set at a crosshead speed of 30 mm/min. Afterwards, fillets were skinned, homogenised and used to determine WHC (Iaconisi et al. 2018) and chemical composition, as described below.

Fillet chemical characteristics and estimation of indices of elongase and desaturase activity

Proximate composition, total lipid and cholesterol contents as well as fatty acid profile were assessed by the method described by AOAC (2012), Folch et al. (1957) and Secci et al. (2018), respectively. To estimate the activities of the enzymes involved in elongation and desaturation of FAs, the ratio of the product to the precursor was calculated (Mattioli et al. 2018). The following equations were utilised:

$$\textit{Thioesterase} = C16/C14$$

$$\textit{Elongase} = C18/C16$$

$$\Delta 9\ \textit{desaturase}\ (16) = \frac{C16:1}{C16:1 + C16} \times 100$$

$$\Delta 9\ \textit{desaturase}\ (18) = \frac{C18:1}{C18:1 + C18} \times 100$$

$$\Delta 9\ \textit{desaturase}\ (16+18) = \frac{C16:1 + C18:1}{C16:1 + C16 + C18:1 + C18} \times 100$$

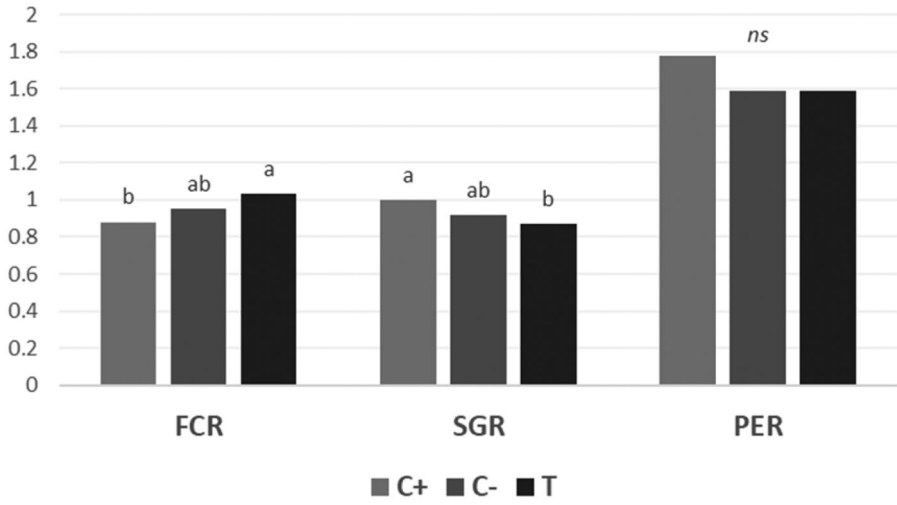

Figure 1. Growth performances of rainbow trout during the feeding trial. a, b as superscript letters indicate significant difference among groups, $p<.05$; *ns*: not significant, $p>.05$; FCR: Feed conversion ratio; SGR: specific growth rate; PER: protein efficiency ratio.

$$\Delta 5+\Delta 6\ desaturase\ (n6) = \frac{C20:2n6+C20:4n6}{C18:2n6+C20:2n6+C20:4n6}\times 100$$

$$\Delta 5+\Delta 6\ desaturase\ (n3) = \frac{C20:5n3+C22:5n3+C22:6n3}{C18:3n3+C20:5n3+C22:5n3+C22:6n3}\times 100$$

Fillet oxidative status

Antioxidant properties, namely ABTS, DPPH, FRAP, and the secondary lipid oxidation products (thiobarbituric acid reactive substances, TBARS) were evaluated on 2 g of homogenised samples according to Mancini et al. (2015) and Vyncke (1970), respectively.

Statistical analysis

Data of the parameters considered at T1 and T2 were analysed with the SAS statistical software (SAS 2007), by a one-way ANOVA followed by a *post hoc* test.

Results

Characterisation of the experimental diets

The colour values and detailed FA profile of the experimental diets are depicted in Supplementary Table S1. Summarising the obtained results, the colour indices seemed to differ between the three experimental diets, specifically, L*, a* and b* were lower in C + in comparison to C − and T; the yellowness index (b*) showed the highest differences between the treatments. The fatty acid profile seemed to be similar between C − and T diets, but deeply different between C + and the other two diets; SFA and PUFAn6 were lower, while PUFAn3 were higher in C + in comparison to C − and T (Table 1), hence, the n3/n6 ratio was higher in C + diet in comparison to C − and T.

Characterisation of the fish flesh at T0 and in vivo performances

At the beginning of the feeding trial, the sampled fish weighed 183.3 ± 29.6 g, were 24.50 ± 1.54 cm long and had a CF value of 0.97 ± 0.07 (mean ± SD). FY, HSI and VSI assumed the values of 63.13 ± 1.89, 0.73 ± 0.09 and 5.44 ± 0.59% whole body weight, respectively. The values of pH, maximum shear force and WHC of the fillets were 6.45 ± 0.09, 29.42 ± 2.00 N, and 92.96 ± 2.06%, respectively. The colour of the skin showed the following values of the colour parameters: L*=54.27 ± 6.87, a*= −1.14 ± 0.70, b*= −0.13 ± 1.78, while the left fillets showed the following values of the colour parameters: L*=52.46 ± 2.24, a*=0.40 ± 0.51, b*=1.98 ± 1.39. Water, ash and crude protein contents were 76.48 ± 1.08, 1.21 ± 0.33 and 19.08 ± 0.38 g/100 g sample, respectively. Cholesterol content was 52.74 mg/100 g of fillet. The FA profile of fillets at T0 is shown in Supplementary Table S2.

During the whole trial, the rearing conditions remained in the optimal range for trout growth, as a matter of fact, the survival rate at the end of the experiment was very high (99.99%) and no relevant differences were noted between the three groups. The mean dissolved oxygen was 9.0 ± 0.49 mg/L and the water temperature fluctuated close to 12.1 ± 0.27 °C

(mean ± SD). The rearing density reached the maximum value of 11.5 kg/m^3 at the end of the trial. A difference in SGR, FCR and weight could be seen at the end of the trial (T2, Figure 1). A declining trend was noted in SGR and FCR going from C+ to T group, being C− intermediate and C+ showing a faster growth and a better FCR than T fish ($p < .05$). The PER was not a discriminating factor between the groups.

Marketable traits and fillet physical characteristics at T1 and T2

After six weeks of feeding with the experimental diets (T1), fish belonging to the different dietary treatments did not show any difference as concerns marketable traits, pH, texture and WHC (data not shown). A longer time period of feeding though, i.e. after six weeks more of feeding (T2), T fish showed the highest and lowest values for FY and HSI, respectively, with a significant difference against the C+ group ($p < .05$), while C− group showed intermediate values for both these parameters (Table 2).

As concerns the colour of the skin and fillets of fish at the end of the 12-week feeding trial (Table 2), the redness index of the skin of T group was lower than that of C− group ($p < .05$), and a pronounced yellow index was registered in C− and T fillets in comparison to C+ ($p < .01$). The difference of colour (ΔE) between the fillets of the different dietary groups showed the following values: $\Delta E_{C+/C-}$ 1.59, $\Delta E_{C+/T}$ 1.94, and $\Delta E_{C-/T}$ 0.95.

Table 2. Growth performances of fish and marketable and physical characteristics of fresh fish and fillets at T2 (12-week long feeding trial).

	C+	C−	T	*p* Value	RMSE
BW at T2, g	474.97^a	442.27^b	416.00^c	<.01	58.62
Standard length, cm	30.33	29.05	29.68	ns	1.05
CF	1.25	1.27	1.18	ns	0.09
FY, %	62.75^b	64.05^{ab}	64.51^a	<.05	1.13
HSI, %	1.27^a	1.15^{ab}	0.99^b	<.05	0.17
VSI, %	8.85	8.01	7.52	ns	1.03
pH	6.42	6.46	6.48	ns	0.05
Maximum Shear Force, N	28.72	28.46	30.13	ns	3.80
WHC, %	92.75	92.56	93.39	ns	2.53
Skin colour					
L*	59.95	59.53	61.74	ns	4.01
a*	−1.57^{ab}	−1.22^a	−1.84^b	<.05	0.39
b*	−0.64	−1.58	−1.28	ns	1.23
Fillet colour					
L*	49.32	48.63	49.42	ns	1.30
a*	−0.35	−0.36	−0.51	ns	0.62
b*	2.92^b	4.60^a	5.20^a	<.01	1.02

BW: whole body weight; C+: positive control; C−: negative control; T: third diet; CF: condition factor; FY: fillet yield; HSI: hepatosomatic index; VSI: viscerosomatic index; WHC: water holding capacity; RMSE: root mean square error.
a, b as superscript letters indicate significant difference among groups; ns: not significant, $p > 0.05$.

Fillet chemical characteristics and estimation of indices of elongase and desaturase activity

Fillet proximate composition did not differ between the three dietary groups both at T1 (data not shown) and at T2 (Table 3); also, cholesterol content of fresh fillets at T2 was similar between the dietary treatments (Table 3).

Fillet fatty acid profile was profoundly affected by the experimental diets as early as after 6 weeks of administration. However, being the fatty acid profile found at T1 akin to the one found at T2, we depict the results obtained at T1 in Supplementary Table S2, while those at T2 are shown in Table 3. Mirroring the fatty acid profile of the diets, C− and T fillets resulted in a significantly higher PUFAn6 content compared to C+, while their PUFAn3 percentage significantly diminished. Consequently, PUFAn3/PUFAn6 ratio also decreased in C− and T groups. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid percentages also mirrored the composition of the diets. Interestingly, the content of C18:3n3 was comparable in C+ and T, while C− showed significantly higher values ($p < .001$). By contrast to the dietary fatty acid profile, SFA content in T fillets was lower than both C+ and C− ($p < .01$). Specifically, T fillets showed the lowest C14:0

Table 3. Chemical composition (g/100g fresh tissue), cholesterol content (mg/100g fresh tissue) and fatty acid profile (g of FAME/100 g total FAME) of fillets at T2 (12-week long feeding trial).

	C+	C−	T	*p* Value	RMSE
Moisture	73.59	73.76	74.35	ns	0.60
Ash	1.28	1.28	1.26	ns	0.03
Crude protein	19.88	20.08	20.18	ns	0.29
Cholesterol	49.66	48.26	48.67	ns	2.83
Total lipids	5.60	4.94	4.49	ns (.0536)	0.72
Fatty acids					
C16:0	13.36^a	13.64^a	12.90^b	<.01	0.33
C18:0	4.605^b	5.13^a	4.720^b	<.05	0.27
C18:1n9	32.44^b	32.85^b	34.14^a	<.05	0.99
C18:2n6	14.49^b	17.84^a	17.11^a	<.001	0.69
C18:3n3	3.38^b	3.74^a	3.41^b	<.001	0.13
C20:5n3	3.33^a	2.02^b	2.21^b	<.001	0.21
C22:6n3	11.79^a	9.18^b	10.00^b	<.01	1.00
sum EPA + DHA	15.13^a	11.20^b	12.22^b	<.01	1.58
SFA	20.59^a	20.80^a	19.56^b	<.01	0.52
MUFA	40.65	39.81	40.95	ns	1.13
PUFAn6	17.48^b	22.19^a	21.57^a	<.001	0.76
PUFAn3	20.67^a	16.73^b	17.46^b	<.001	1.17
PUFAn3/PUFAn6	1.18^a	0.75^b	0.81^b	<.001	0.07

The following FAs were used for calculating the classes of FAs but they are not listed because below 3% of total FAME: C12, C13, C14, C14:1n5, C15, C15iso, C15anteiso, C16iso, C16:1n7, C16:1n9, C16:2n4, C16:3n4, C16:4n1, C17, C17:1, C18:1n7, C18:2n4, C18:3n6, C18:3n4, C18:4n1, C20, C20:1n11, C20:1n9, C20:1n7, C20:2n6, C20:4n6, C20:3n3, C20:4n3, C22, C22:1n7, C22:1n11, C22:2n6, C21:5n3, C22:4n6, C22:5n6, C22:5n3, C24.
RMSE: root mean square error; C+: positive control; C-: negative control; T: third diet; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
a, b as superscript letters indicate significant difference among groups; ns: not significant, $p > 0.05$.

Table 4. Estimated indices of lipid metabolism in fresh fillet at T2 (12-week long feeding trial).

	C+	C−	T	*p* Value	RMSE
Thioesterase	7.94[b]	10.81[a]	10.78[a]	<.0001	0.41
Elongase	0.34[b]	0.37[a]	0.36[ab]	<.05	0.01
Δ9 desaturase (C16)	17.46[a]	14.70[b]	14.55[b]	<.0001	0.90
Δ9 desaturase (C18)	88.32[a]	87.26[b]	88.53[a]	<.001	0.47
Δ9 desaturase (C16 + C18)	67.67[b]	66.63[c]	68.67[a]	<.0001	0.57
Δ5 + Δ6 desaturase n6	9.86[b]	10.88[a]	11.44[a]	<.01	0.72
Δ5 + Δ6 desaturase n3	82.24[a]	75.52[c]	78.73[b]	<.0001	1.71

RMSE: root mean square error; C+: positive control; C-: negative control; T: third diet.
a, b, c as superscript letters indicate significant difference among groups; ns: not significant, $p > 0.05$.

($p < .001$, not shown) and C16:0 ($p < .01$) contents in comparison to both C + and C−. Additionally, C18:0 contents in C + and T were analogous, while C − displayed significantly higher values ($p < .05$) despite this FA was found higher in the T diet than in C − and C + ones.

The results of the estimated indices of lipid metabolism are depicted in Table 4. Significant differences between the three dietary groups were revealed for all indices. Taking C − and T into consideration, the estimated activities of Δ9 desaturase (C18), Δ9 desaturase (C16 + C18) and Δ5 + Δ6 desaturase n3 were higher in T as compared to C− ($p < .001$, $p < .0001$, $p < .0001$, respectively). On the other hand, the estimated activities of thioesterase, elongase, Δ9 desaturase (C16) and Δ5 + Δ6 desaturase (n6) enzymes were similar between C − and T groups ($p > .05$). Overall, T showed the highest Δ9 desaturase (C18), Δ9 desaturase (C16 + C18) and Δ5 + Δ6 desaturase n6 activities.

Oxidative status

As summarised in Table 5, parameters of the antioxidant capacity of fillets from fish fed for 12 weeks with the experimental diets had different trends. Specifically, ABTS and DPPH showed similar values between the groups differently fed, while FRAP value of the T group was significantly lower than that of C + group ($p < .01$), while C − group displayed intermediate value. TBARS assay indicated that the different diets affected the lipid peroxidation in fillets, showing that C − and T fillets were less prone to peroxidation in comparison to C+ ($p < .05$).

Table 5. Oxidative status of fresh fillets at T2 (12-week long feeding trial). ABTS, DPPH, and FRAP values are expressed as mmol Trolox-eq/kg fillet; TBARS values are expressed as mg MDA-eq/kg fillet.

	C+	C−	T	*p* Value	RMSE
ABTS	0.41	0.43	0.40	ns	0.09
DPPH	0.10	0.13	0.12	ns	0.01
FRAP	0.10[a]	0.09[ab]	0.09[b]	<.01	0.00
TBARS	0.50[a]	0.30[b]	0.29[b]	<.05	0.10

RMSE: root mean square error; C+: positive control; C-: negative control; T: third diet; ABTS: 2,20-azinobis(3-ethylbenzthiazoline-6-sulphonic acid); DPPH; 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; TBARS: thiobarbituric acid reactive substances.
a, b as superscript letters indicate significant difference among groups; ns: not significant, $p > .05$.

Discussion

Fish performances, marketable and physical characterisation

The present study showed that the 5% dietary inclusion of a commercial blend of seaweeds tended to reduce rainbow trout growth performance. Differences were unveiled as early as after 6 weeks of feeding when it comes to colour of skin and flesh and to the FA profile of fillets. Additional six weeks of feeding did not further change most of the parameters, although growth performance declined in T fish. In spite of the worsened growth performance, marketable traits could be considered acceptable for this species. For this reason, it seems reasonable to use macroalgae for a short period of time, for instance in finishing diets.

As a result of T diet administration, HSI decreased and FY increased ($p < .05$) as compared to C+. It is commonly accepted that HSI provides an indication on the status of energy reserves and on the general metabolic activity. Researchers mainly noticed an increase in HSI following a decrease in feed availability, especially while studying seasonal variation of this index, indicating that the energy usually allocated to tissue growth is destined to fight against stressors (Craig et al. 2000; Singh and Srivastava 2015). Focussing on the impact of seaweed administration on fish HSI, Soler-Vila et al. (2009) found that the HSI value decreased in rainbow trout fed for 12.5 weeks while increasing the substitution level of fishmeal and wheat starch; specifically, decreasing trend was apparent and became significant at 10 or 15% dietary inclusion of the red alga *Porphyra dioica*. More recently, the incorporation of 5%, 15% or 25% of *Gracilaria cornea* or *Ulva rigida* meal in feed for sea bream (*Sparus aurata)* juveniles was tested after 70 days of administration (Vizcaíno et al. 2016); results showed an inverse relationship between dietary inclusion of seaweeds and HSI value, moreover, the HSI was significantly lower, compared to control, starting from the 5% inclusion level of both seaweeds. In the present study, the lowering of HSI, together with the reduced PER, could suggest the difficulty of fish to efficiently use feed components and metabolised energy. In this regards, a recent study carried out by Sotoudeh and

Mardani (2018) on rainbow trout fry showed a diminished protease activity with increasing dietary *Gracilaria pygmaea* levels in feed. This outcome is in general agreement with Vizcaíno et al. (2016), who reported that the proteolytic activities in *S. aurata* fed *Ulva*- or *Gracilaria*-supplemented diets were lower than those of gilthead sea bream fed with the control diet.

Another possible explanation of the decreased HSI might be a reduced deposition of lipid in the liver, as previously found in *O. mykiss* fed on a diet containing 10% *Ulva* meal (Güroy et al. 2013). Supporting this assertion, Dantagnan et al. (2009) highlighted that, in *O. mykiss* juveniles, the lipid utilisation can be enhanced by macroalgae meal in diets, thus resulting in a lower amount of lipid deposition in tissues than in the no-added diet group. In agreement with Dantagnan et al. (2009), a marginal effect ($p = .053$) on fillet total lipids was found in the present study. Focussed studies on the effect of seaweeds on liver are warmly encouraged given the central role of this organ in macronutrient metabolism.

Flesh pigmentation is one of the major quality attributes of salmonids (de Francesco et al. 2004) and it can be easily evaluated by instrumental analysis, which usually well reflects carotenoid deposition and concentration (Wathne et al. 1998). Carotenoids, such as β-carotene, astaxanthin and yellow xanthophylls, especially lutein and zeaxanthin, are mainly responsible for flesh colouration. These molecules are widespread both in terrestrial vegetables and seaweeds (Wells et al. 2017). For this reason, in the present study, the variation of colour in fish tissues could be attributed to the different proportions of the vegetable ingredients in the experimental diets as well as to the seaweed inclusion. In this regard, the colour values that were similar between C − and T diets, which both resulted in higher a* and b* values than those of C + diet, supported the idea that the vegetal ingredients were the main source of pigments. These differences also corroborated results obtained for the skin and the muscle of fish, where only a slight difference in the a* value of the skin emerged between C − and T. Overall, a* and b* indices of the skin and of the muscle of trout were significantly affected by the dietary treatments, thus confirming the findings of Araújo et al. (2016), who showed that different pigments preferentially deposit in skin or muscle. Indeed, rainbow trout skin was found to be the principal accumulation site for β-carotene, positively correlated with a* index, while muscle mainly stores lutein and zeaxanthin, whose concentrations are positively correlated with b* value (Araújo et al. 2016).

Colour differences (ΔE) underlined that no difference in colour could be perceived between C − and T ($\Delta E < 1$), while C + differed from both C − and T ($1 < \Delta E < 3.5$) (Mokrzycki and Tatol 2011). In summary, the present results revealed that 5% soybean substitution with a commercial blend of seaweeds, in a vegetable rich diet, is not enough to deeply modify skin and muscle colourations of trout, nor to convey a redder flesh pigmentation, as claimed by Araújo et al. (2016) and Soler-Vila et al. (2009).

Recently, authors have found that changes in the dietary protein sources, such as type and quantity, did not affect textural attribute of rainbow trout fillets. Indeed, Borgogno et al. (2017) verified that the Warner-Bratzler shear force registered for rainbow trout muscle was unaffected with increasing substitution levels of fishmeal with insect meal, however, the performed sensory analysis revealed that differences between dietary treatments were perceived by trained panellist in terms of fillet textural attributes (tenderness, juiciness, fibrousness). Since no effect of the experimental diet on shear force emerged in the present trial, further studies would be useful to understand if texture might be perceived as different by a trained panel or if liking judgements of fillets by consumers might be altered by the composition of the feed utilised for feeding the fish, as found by Bruni et al. (2020).

Fillet chemical composition and estimated lipid metabolism enzymatic activities

Concerning the overall proximate composition of the fillets, the absence of a significant effect of the diet aligns with previous studies that considered a 5% inclusion level (dry matter) of *Gracilaria vermiculophylla* and *Porphyra dioica* in diets for rainbow trout (Soler-Vila et al. 2009; Araújo et al. 2016), *Gracilaria cornea* and *Ulva rigida* in diets for gilthead sea bream juveniles (Vizcaíno et al. 2016), and *Sargassum horneri* in diets for juvenile turbot (*Scophthalmus maximus*) (Wang et al. 2019). Nevertheless, the lower lipid content in our T samples was of notice, since a numerical difference near to the significant threshold was registered (the calculated *p*-value was .054). In literature, the lipid source and its level of inclusion in aquafeeds are the most reported modulators of lipase activity (Morais et al. 2004), but the addition of different seaweed species at various concentrations also seemed to modulate the activity of this enzyme (Peixoto et al.

2016). Indeed, intestinal lipase activity of European sea bass (*Dicentrarchus labrax*) fed diets supplemented with no seaweed or with *Gracilaria* spp., *Ulva* spp., and *Fucus* spp. at 2.5 or 7.5% and a mix of the three (each at 2.5% inclusion percentage) for 84 days was not significantly different between the different dietary treatments groups except for the fish fed *Ulva* spp., which had the lowest lipase activity between all the treatments, with a significant difference compared to fish fed the mix of the three seaweeds. These results encourage researchers to enrich the literature with additional investigations on the effects of macroalgae on rainbow trout lipid deposition.

It is a matter of fact that the level of PUFAn3 in the fillets of farmed fish has dramatically declined in the recent decades due to the replacement of PUFAn3-rich fish oil and fish meal with vegetable sources rich in MUFA and PUFAn6 (Storebakken et al. 2000). In this regard, the use of seaweeds, another marine source rich in PUFAn3 (Wells et al. 2017), could theoretically counteract the high MUFA and PUFAn6 content of vegetable sources contained in feed ingredients currently utilised. As exposed in Table 1, PUFAn6 and SFA mainly represented the fatty acid composition of C − diet. Moreover, the analogy between the fatty acid profiles of C − and T diets showed that the 5% inclusion of the commercial blend of seaweeds was not enough to induce a substantial modification of the fatty acid composition of the diet, especially concerning the PUFAn3 content.

Nevertheless, recent studies have enquired the possibility that dietary seaweeds enhance PUFAn3 in fish muscle (Dantagnan et al. 2009; Güroy et al. 2013; Wilke et al. 2015). Although the fillet FA profile results did not manifestly back up this hypothesis, the estimated indices of lipid metabolism suggested that the commercial seaweed blend modulated the activity of certain enzymes. The Δ9 desaturase activity on stearic acid was significantly higher in T fish in comparison to C − fish ($p < .001$). It seems also noteworthy to comment on the Δ5-Δ6 desaturase activity carried out on the n3 series, namely, the index increased in the T group in comparison to the C − group ($p < .0001$). The differences between C − and T groups suggest that the enzymes involved in the desaturation of long chain and very long chain FAs were modulated by the dietary inclusion of seaweeds. As expected, the estimated metabolic indices were significantly different between C + and C − and this is easily explained by revising dietary FA composition. In fact, the considerable difference in FA profile between of C − and C + diets likely induced a regulation of fish elongase and desaturase enzymatic activities, as well documented in other studies on Atlantic salmon where diets with different amounts of PUFAn3 were compared (Stubhaug et al. 2005; Turchini and Francis 2009).

Noticeably, lipid metabolism was thought to modulate SFA content in fillets as some slight divergence between feed and fillet FA profiles was found regarding SFA. While the T diet contained the same amount of SFA as the C − diet, this was not the case for the fillets from fish fed with these diets. Despite the paucity of research highlighting a decrease of SFA content in fish fed with algae, experiments on rats fed with ethanolic extracts of *Ecklonia stolonifera* (Yoon et al. 2008) or with fucoidan polysaccharide sulphuric acid ester from *Laminaria japonica* Aresch (Huang et al. 2010) registered a significant reduction in triglycerides (TGs) in hyperlipidaemic rat plasma. In addition, an *in vitro* study supported this thread, observing that polysaccharides extracted from different algae induced hypolipidemic activity on human cell lines insignificantly different as compared to the reference drug Fluvastatin (Matloub et al. 2015). We should remind that *Ulva* spp. lowered the activity of lipase (Peixoto et al. 2016), which is responsible for TG and not phospholipid hydrolysis. Since TGs, in comparison to phospholipids, generally contain a lower proportion of PUFA and a higher proportion of SFA, the reduction of SFA in the total lipid extract of fillets could be explained by a possible reduction in lipase activity induced by the dietary seaweed blend. However, more studies about the lipid metabolism of fish fed seaweeds are strongly suggested.

Fillet oxidative status

Finally, there is a very broad literature on marine algae as source of antioxidant compounds (Wells et al. 2017), which play an important role in preventing lipid oxidation by acting as radical scavengers, chelating agent, etc. In the present study, the similarities of the results on the total antioxidant capacity (monitored by ABTS, DPPH, and FRAP assays) and lipid peroxidation products (TBARS) between fillets belonging to the T and C − groups suggested that T diet did not carry a specific antioxidant ability in comparison to the C − diet. This result goes in the same direction as previous studies that did not find differences in hepatic TBARS levels of sea bass juveniles fed 6 practical diets supplemented either with or without *Gracilaria* spp., *Ulva* spp. or *Fucus* spp. at 2.5 or 7.5% levels, or a mix of the three seaweeds (each supplemented at 2.5%)

(Peixoto et al. 2016). Moreover, Sotoudeh and Mardani (2018) have recently quantified TBARS content in the liver of rainbow trout fry fed diets supplemented with 0, 30 and 60 g/kg of *Gracilaria pygmaea*, finding that it was not significantly affected by the different diets.

Conclusions

Seaweeds are a promising source of not only nutritive but also nutraceutical compounds and research in this field should be encouraged to delve into the effects of different algal species on fish metabolism. The present study showed that the majority of marketable and physical parameters of rainbow trout fed with a low dietary inclusion level of a commercial blend of seaweeds was not negatively affected, while fatty acid profile is more of a concern. On the other hand, the performed study highlighted an interesting lowering effect on SFA content and an increase in the estimated desaturase activity. As rainbow trout is renowned to be able to modulate its FA composition, studies in this specific field are highly encouraged to diminish the marine ingredients allocated to aquafeeds and thus allow a sustainable development of the aquaculture sector.

Ethical approval

The present trial was performed according to the European Directive 2010/63/EU . The protocol utilized in this experiment was approved by the Italian Ministry of Health (authorization n. 120/2008-A).

Disclosure statement

The authors declare no conflict of interest.

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