Aquacult Int (2013) 21:605–615
DOI 10.1007/s10499-012-9592-7

Effect of autoclaved *Ulva* meal on growth performance, nutrient utilization and fatty acid profile of rainbow trout, *Oncorhynchus mykiss*

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Received: 16 December 2011 / Accepted: 30 August 2012 / Published online: 26 September 2012

Abstract A feeding trial was conducted to investigate the effects of the dietary incorporation of autoclaved and raw (non-autoclaved) *Ulva* meal on juvenile rainbow trout (*Oncorhynchus mykiss*) growth, nutrient utilization, body composition, diet digestibility and fatty acid composition. An algae-free control diet and four experimental diets with either 5 or 10 % inclusion levels of raw *Ulva* meal (5 % = RU5; 10 % = RU10) or autoclaved *Ulva* meal (5 % = AU5; 10 % = AU10) were formulated. Fish were fed three times daily to apparent satiation for 12 weeks. The growth of fish fed RU10 (final weight = 76.7 ± 3.31 g; SGR = 2.8 ± 0.05 %) diet was significantly higher than that in fish fed the AU10 diet (67.5 ± 1.61 g; SGR = 2.6 ± 0.03 %). The feed conversion ratio (FCR) was significantly better in fish fed the RU10 diet (0.9 ± 0.06) compared with control diet (1.0 ± 0.06). Condition factor, viscerosomatic index and dress-out remained unaffected by dietary treatment. The hepatosomatic index (HSI) was significantly lower ($P < 0.05$) in fish fed the AU10 diet (1.2 ± 0.19) than fish fed the other diets (≥1.6). The level of eicosapentaenoic acid (20:5n-3—EPA) in muscle from fish fed the RU10, AU5 and AU10 diets was significantly higher than in fish fed control diet ($P < 0.05$). Dietary inclusion of *Ulva* meals resulted in a significant increase in muscle docosapentaenoic acid (22:5n-3—DPA) levels at the end of the feeding period. The results indicate that the dietary inclusion of raw *Ulva* meal at levels of up to 10 % can be used without significant negative effects on the growth performance, nutrient utilization, dietary digestibility and muscle fatty acid composition. In fact, the inclusion of dietary raw and autoclaved *Ulva* meal elevated n-3 fatty acids levels of rainbow trout muscle.

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Keywords *Ulva* meal · Feed additive · Algal products · Heat treatment · Salmonids · Fatty acid

Introduction

Fishmeal is traditionally the main protein source used in fish diets because it is a rich source of essential amino acids, essential fatty acids, energy and minerals (Hertrampf and Piedad-Pascual 2000). In the aquaculture industry, the increasing cost and inadequate supply of fishmeal limit sustainability. Therefore, reducing fishmeal inclusion levels and replacing fishmeal with cost-effective, widely available and sustainable feedstuffs are considered essential for the future development of the aquaculture industry (Tacon et al. 2006; Gatlin et al. 2007; Kaushik and Hemre 2008). Many alternative plant feedstuffs contain high levels of deleterious carbohydrate fractions, complex fiber-type components and anti-nutritional factors (ANF), which can limit their use in carnivorous fish diets (Drew et al. 2007; Krogdahl et al. 2010).

Macroalgae meals have emerged as interesting candidate ingredients for low inclusion level in aquafeeds (Valente et al. 2006; Dantagnan et al. 2009; Soler-Vila et al. 2009; Güroy et al. 2011). Enhancements of growth, feed utilization, lipid metabolism, physiological activity, stress response, disease resistance and carcass quality have been reported with low-level dietary algal inclusion (Mustafa et al. 1994, 1995, 1997; Mustafa and Nakagawa 1995; Nakagawa 1997; Nakagawa and Montgomery 2007). Of these algae, *Ulva* sp. are among the most commonly used as they are a good source of protein, vitamins, pigments and minerals (Garcia-Casal et al. 2007; Ortiz et al. 2006). The effects of dietary *Ulva* on fish growth performance, nutrient utilization and immune response have been investigated for several fish species, including rainbow trout (*Oncorhynchus mykiss*) (Yildirim et al. 2009; Güroy et al. 2011), tilapia (*Oreochromis niloticus*) (Güroy et al. 2007; Azaza et al. 2008; Ergun et al. 2009), common carp (*Cyprinus carpio*) (Diler et al. 2007) and the European sea bass (*Dicentrarchus labrax*) (Valente et al. 2006).

However, no information is available regarding the efficacy of using autoclaved *Ulva* meal in rainbow trout diets. The objective of the present study was to evaluate the effect of dietary raw and autoclaved *Ulva* meal on rainbow trout on the growth performance, nutrient utilization, body composition, digestibility and muscle fatty acid profile.

Materials and methods

Diet formulations

Ulva rigida was freshly collected from the near-shore waters of the Dardanelles, Canakkale, Turkey. Algal samples were thoroughly washed with sea water, dried at 40 °C for 48 h and fine-milled with a laboratory blender to produce raw *Ulva* meal. Fresh *U. rigida* was autoclaved (121 °C at 1.0 kg cm^{-2} for 30 min), dried at 40 °C for 48 h and ground to produce the autoclaved *Ulva* meal. An algae-free control diet and four experimental diets, with either 5 or 10 % inclusion levels of raw *Ulva* meal (5 % = RU5; 10 % = RU10) or autoclaved *Ulva* meal (5 % = AU5; 10 % = AU10), were formulated. The formulation and chemical composition of the experimental diets are displayed in Table 1. The dietary fatty acid composition is shown in Table 2. Feed ingredients were

Table 1 Formulation and proximate composition of experimental diets

	C	RU5	RU10	AU5	AU10
Fish meal[a]	50.0	50.0	50.0	50.0	50.0
Ulva meal[b]	–	5.0	10.0	–	–
Autoclaved *Ulva* meal[b]	–	–	–	5.0	10.0
Soybean meal[c]	24.5	24.5	24.5	24.5	24.5
Wheat feed[d]	13.0	8.0	3.0	8.0	3.0
Fish oil[e]	10.0	10.0	10.0	10.0	10.0
Vitamin premix[f]	0.9	0.9	0.9	0.9	0.9
Mineral premix[g]	0.6	0.6	0.6	0.6	0.6
Celite	1.0	1.0	1.0	1.0	1.0
Chemical composition					
Crude protein (%)	45.0	45.2	45.2	45.4	45.9
Crude lipid (%)	15.1	15.0	15.0	15.0	14.9
Crude ash (%)	9.1	11.6	11.4	11.1	10.8
Crude fiber (%)	1.8	2.0	2.2	1.9	2.1
Nitrogen-free extract (%)[h]	29.0	26.2	26.2	26.6	26.3

Dietary codes: C = control, RU5 = 5 % raw *Ulva* inclusion, RU10 = 10 % raw *Ulva* inclusion, AU5 = 5 % autoclaved *Ulva* inclusion, AU10 = 10 % autoclaved *Ulva* inclusion

[a] Anchovy fish meal. Can Kardesler Fish Meal Corporation, Samsun, Turkey

[b] Dardanelles

[c] Abide Feed Mill Company, Canakkale, Turkey

[d] Kepez Un, Canakkale, Turkey

[e] Anchovy fish oil. Can Kardesler Fish Meal Corporation, Samsun, Turkey

[f] Per g mixture: vitamin A: 342 IU; vitamin D_3: 329 IU; vitamin E: 0.0274 IU; vitamin K_3: 5.48 mg; vitamin B_1: 2.05 mg; vitamin B_2: 3.42 mg; vitamin B_3: 20.5 mg; vitamin B_5: 5.48 mg; vitamin B_6: 2.05 mg; vitamin B_{12}: 2.74 mg; vitamin C: 24.0 mg, Kartal Chemical Incorporated, Kocaeli, Turkey

[g] Per g mixture: biotin: 0.411 mg; folic acid: 0.685 mg; Zn: 12.3 mg; Mn: 4.80 mg; Cu: 1.64 mg; I: 0.274 mg; Se: 0.0274 mg; Ca: 125 mg; K: 189 mg, Kartal Chemical Incorporated, Kocaeli, Turkey

[h] Nitrogen-free extracts (NFE) = 100 − (crude protein + crude lipid + crude ash + crude fiber)

thoroughly mixed with the addition of oil and a vitamin/mineral premix using a laboratory food mixer. The moistened mixture was pelleted (2 mm pellet size) in a meat grinder and dried in a forced air oven (40 °C) until moisture content was reduced to approximately 10 %. The diets were stored in sealed plastic bags at −20 °C prior to use.

Experimental facility and fish

Juvenile rainbow trout, *O. mykiss*, were transported from a local fish farm (Keskinler Trout Farm, Bayramic, Canakkale, Turkey) and acclimatized to laboratory conditions at the Fish Nutrition Aquarium Unit of Canakkale Onsekiz Mart University, Faculty of Fisheries, for 4 weeks while being fed a commercial diet (Bagci Feed Company, crude protein: 50 %, crude lipid: 18 %). Three hundred fish (∼7 g) were randomly allocated into 150-l circular tanks (20 fish per tank) within a recirculation freshwater system. Each treatment was conducted in triplicate tanks. Fish were fed to apparent satiation three times a day (08:00, 12:00 and 16:00) for 12 weeks. During the experimental period, temperature was maintained at 16 ± 1.5 °C and dissolved oxygen levels at >80 % saturation with a 12:12 h

Table 2 Fatty acid composition of experimental diets (% of total fatty acids)

	C	RU5	RU10	AU5	AU10
14:0	5.3	5.3	5.7	5.2	5.8
15:0	0.9	0.8	0.9	0.7	0.9
16:0	21.3	20.7	21.7	20.8	22.8
17:0	0.9	0.8	0.8	0.7	1.0
18:0	4.7	4.2	4.4	4.2	4.8
20:0	1.1	0.8	1.0	0.8	1.2
∑Saturated[a]	34.2	32.5	34.6	32.3	36.5
18:1n-9	18.3	17.8	17.7	17.9	19.8
18:1n-7	2.0	2.4	2.5	2.7	3.5
20:1n-9	1.3	1.2	1.2	1.2	1.3
∑Monounsaturated[b]	21.6	21.4	21.3	21.8	24.6
18:2n-6	5.5	5.5	5.3	4.8	5.2
18:3n-6	0.4	0.2	0.2	0.2	0.3
20:3n-6	0.1	0.1	0.1	0.1	0.1
20:4n-6	0.2	0.2	0.1	0.1	0.3
18:3n-3	1.5	1.6	1.7	1.5	1.4
18:4n-3	1.4	2.0	1.6	2.0	1.3
20:3n-3	0.8	0.8	0.8	0.7	0.9
20:5n-3	8.0	9.4	8.6	10.5	9.0
22:5n-3	0.8	1.0	0.8	1.0	0.9
22:6n-3	16.4	17.5	16.3	18.2	17.9
∑Polyunsaturated[c]	35.0	38.2	35.5	39.1	37.2
∑ n-6	6.2	5.9	5.7	5.2	5.9
∑ n-3	28.9	32.3	29.7	33.9	31.2

Dietary codes: *C* = control, RU5 = 5 % raw *Ulva* inclusion, RU10 = 10 % raw *Ulva* inclusion, AU5 = 5 % autoclaved *Ulva* inclusion, AU10 = 10 % autoclaved *Ulva* inclusion

[a] Includes: 21:0, 22:0, 23:0, 24:0

[b] Includes: 14:1, 15:1, C16:1, C17:1, 22:1n-9

[c] Includes: 18:4n-3, 20:3n-6

light:dark photoperiod. Water flow rate was approximately 12 l min^{-1} $tank^{-1}$. Fish in each tank were individually weighed at the start and end of the feeding trial. Fish standard length (SL) was measured with fish measuring boards with 1-mm divisions at the start and end of the feeding trial. Daily feed intake was recorded, and each tank was weighed every 2 weeks to observe the growth and feed utilization parameters.

Sampling and proximate composition

Six fish were randomly taken from the initial pool of fish at the beginning of the experiment, and three fish from each tank (nine fish per treatment) were sampled at the end of the trial to determine whole body proximate analysis. At the end of the feeding trial, all fish were starved for 48 h to ensure that the digestive tract was devoid of feed. Analysis of crude protein, moisture, fiber and ash in the whole body of fish and the diets was performed according to standard AOAC (2000) procedures. Dietary and whole-body lipids were extracted according to

the procedure of Folch et al. (1957) with chloroform/methanol (2:1 v/v). Nitrogen-free extract (NFE) was calculated by taking the sum values for crude protein, lipid, ash and crude fiber and then subtracting this value from 100. The gross energy content of the diets and fish was calculated using the conversion factors of 23.7 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid and 17.2 kJ g^{-1} for carbohydrate (Brett and Groves 1979).

Samples of muscle from three fish per each tank were taken for fatty acid analyses. The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14 % boron trifluoride–methanol (AOAC 2000). Fatty acid methyl esters (FAME) were analyzed using a flame ionization gas chromatograph (Shimadzu GC-2014) equipped with an Omegawax 250 capillary column (30 m L X 0.25 mm internal diameter), a flame ionization detector (FID) and a split injection system with nitrogen carrier gas. The injector port and detector temperatures were maintained at 250 and 260 °C, respectively. The column temperature program was held at 140 °C for 5 min and then elevated at a rate of 3 °C/min to 200 °C. Total run time was 60 min per sample. Fatty acids were identified by comparing their retention times to that of fatty acid standards (Sigma-Aldrich Co, USA).

Fish performance and somatic indices

Fish growth performance and nutrient utilization were calculated according to the following formulae:

$$\text{Feed conversion ratio (FCR)} = \text{FI}/\text{WG}$$
$$\text{Specific growth rate (SGR)}\ (\%) = 100((\ln \text{FBW} - \ln \text{IBW})/T)$$
$$\text{Protein efficiency ratio (PER)} = \text{WG}/\text{PI}$$
$$\text{Net protein utilization (NPU)}\ (\%) = 100(\text{PG}/\text{PI})$$

Six fish were randomly taken from the initial pool at the beginning of the experiment, and three fish from each tank (nine fish per treatment) were randomly sampled at the end of the trial to determine somatic indices.

The somatic indices were calculated according to the following formulae:

$$\text{Condition factor (K)} = 100(\text{FBW}/(\text{SL}^3))$$
$$\text{Dress}-\text{out (DO)}\ (\%) = 100((\text{FBW} - \text{VW})/\text{FBW})$$
$$\text{Hepatosomatic (HSI)}\ (\%) = 100(\text{LW}/\text{FBW})$$
$$\text{Viscerosomatic (VSI)}\ (\%) = 100(\text{VW}/\text{FBW})$$

where FBW is the final body weight (g), IBW is the initial body weight (g), FI = feed intake (g), WG = weight gain (g), T = time (days), PI = dietary protein intake (g), PG = protein gain (g), EG = energy gain, EI = dietary energy intake, SL = standard length (cm), LW = liver weight (g) and VW = viscera weight (g).

Digestibility trial

The digestibility trial was immediately conducted at the end of the growth trial. Feces were collected from each fish within each tank using stripping techniques. Fish were fed in excess by hand 10 h before stripping. Manual stripping of fish was accomplished by netting and anesthetizing all fish in the tank. The feces were then removed from the distal intestine using gentle abdominal pressure. Care was taken to exclude urinary excretions or

mucus from the collection. Wet fecal samples were then dried at 50 °C (48 h) before analysis. All diets contained Celite (1 g kg^{-1}) as the indigestible marker. Analysis of acid-insoluble ash in diets and feces was performed according to standard AOAC methodology (2000).

Digestibility coefficients of nutrients were determined with the following formula:

$$\text{ADC}\% = 100 \times [1 - (\text{marker in diet}/\text{marker in feces}) \times (\text{nutrient in feces}/\text{nutrient in diet})]$$

Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) and post hoc LSD using the statistical software package Statgraphics 7.0 (Manugistics Incorporated, Rockville, MD, USA) (Zar 2001). All percentage data and ratios were arcsine transformed before being subjected to the analysis. The results were treated statistically significant at the 5 % level.

Results

The overall growth performance and nutrient utilization data are displayed in Table 3. The mean weights of fish at the end of the 12-week feeding trial ranged from 76.7 g (RU10 diet) to 67.5 g (AU10 diet) (Fig. 1). The mean weight and specific growth rate (SGR) of fish fed diet RU10 were significantly higher than fish fed the AU10 diet. However, there were no significant differences between any of the remaining treatments (Table 3). The feed conversion ratio (FCR) of fish fed the RU10 diet was significantly lower than fish fed the control diet (Table 3). Although the highest protein efficiency ratio (PER) was recorded in fish fed the RU10 diet, no significant differences were observed among the groups (Table 3). Net protein utilization (NPU) of fish fed AU5 was significantly lower than fish fed the control, RU5 and RU10 diets ($P > 0.05$). Furthermore, feed intake (FI) was significantly lower for AU5, AU10 and RU10 fed fish than fish fed the control or RU5 diets.

Table 3 Growth performance and nutrient utilization of rainbow trout fed the experimental diets at the end of 12-week feeding trial

	C	RU5	RU10	AU5	AU10
Initial mean weight (g)	7.9	7.9	7.9	7.9	7.9
Final mean weight (g)	72.0 ± 3.5^{ab}	70.0 ± 2.5^{ab}	76.7 ± 3.3^{b}	71.6 ± 4.2^{ab}	67.5 ± 1.6^{a}
Specific growth rate (SGR; % day^{-1})	2.7 ± 0.1^{ab}	2.7 ± 0.1^{ab}	2.8 ± 0.1^{b}	2.7 ± 0.1^{ab}	2.6 ± 0.1^{a}
Feed conversion ratio (FCR)	1.0 ± 0.1^{b}	1.0 ± 0.1^{ab}	0.9 ± 0.1^{a}	0.9 ± 0.1^{ab}	0.9 ± 0.1^{ab}
Feed intake (FI; %)	2.0 ± 0.1^{b}	2.0 ± 0.1^{b}	1.8 ± 0.1^{a}	1.8 ± 0.1^{a}	1.8 ± 0.1^{a}
Protein efficiency ratio (PER)	2.2 ± 0.1	2.2 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.3 ± 0.1
Net protein utilization (NPU%)	40.2 ± 2.7^{b}	40.7 ± 2.3^{b}	43.5 ± 2.0^{b}	35.2 ± 2.9^{a}	39.1 ± 2.4^{ab}

Dietary codes: C = control, RU5 = 5 % raw *Ulva* inclusion, RU10 = 10 % raw *Ulva* inclusion, AU5 = 5 % autoclaved *Ulva* inclusion, AU10 = 10 % autoclaved *Ulva* inclusion. $n = 3$

In the same line, values with different superscript letters are significantly different ($P < 0.05$). Data are expressed as mean ± SD

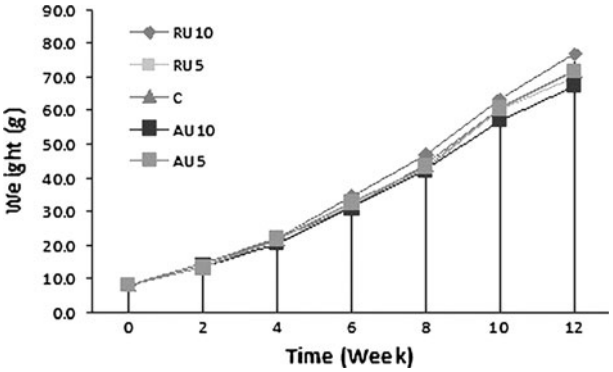

Fig. 1 The mean weight of rainbow trout fed on experimental diets during the 12 week feeding trail

The apparent digestibility coefficients (ADCs) of fish fed the experimental diets are displayed in Table 4. The ADCs of protein (ranging from 93.45 to 98.94 %) were significantly affected ($P < 0.05$) by the various dietary treatments. The protein ADCs of fish fed *Ulva* diets were significantly higher than fish fed the control diet. No significant differences were observed in the ADCs of lipid among dietary treatments ($P > 0.05$).

Data on biometric parameters of fish fed the different diets are displayed in Table 5. Aside from the hepatosomatic index (HSI), which was significantly lower ($P < 0.05$) in fish fed the AU10 diet than in fish fed the other diets, no differences were observed between the groups. The viscerosomatic index (VSI) of fish fed diets AU5 and AU10 was significantly higher than the fish fed the other diets. Inversely, this trend was observed with dress-out (DO).

The whole-body composition of fish fed the experimental diets is presented in Table 6. Fish fed AU5 contained a significantly lower ($P < 0.05$) protein content than fish fed the other diets. Furthermore, the body protein content of fish fed the control and RU5 diets was significantly higher than fish fed the AU5 and AU10 diets.

The muscle fatty acid composition of rainbow trout is shown in Table 7. The level of eicosapentaenoic acid (20:5n-3—EPA) of fish fed RU10, AU5 and AU10 diets was significantly higher when compared to fish fed the control diet ($P < 0.05$). Dietary inclusion of *Ulva* meals resulted in a significant increase of docosahexaenoic acid (22:6n-3—DHA) in the muscle at the end of the feeding period. The n-3 fatty acids level of the groups fed dietary *Ulva* meal was significantly higher ($P < 0.05$) than the control group.

Table 4 Apparent digestibility coefficients of rainbow trout fed the experimental diets at the end of 12-week feeding trial

%	*C*	RU5	RU10	AU5	AU10
Protein	93.5 ± 0.6^{a}	99.0 ± 0.5^{b}	98.2 ± 0.8^{b}	97.4 ± 0.6^{b}	96.4 ± 0.6^{b}
Lipid	95.7 ± 0.5	98.5 ± 0.6	97.4 ± 0.5	97.9 ± 0.9	98.3 ± 0.7

Dietary codes: C = control, RU5 = 5 % raw *Ulva* inclusion, RU10 = 10 % raw *Ulva* inclusion, AU5 = 5 % autoclaved *Ulva* inclusion, AU10 = 10 % autoclaved *Ulva* inclusion. $n = 3$

In the same line, values with different superscript letters are significantly different ($P < 0.05$). Data are expressed as mean ± SD

Table 5 Biological indices of rainbow trout fed the experimental diets at the end of 12-week feeding trial

	C	RU5	RU10	AU5	AU10
Condition factor (CF)	1.8 ± 0.2	1.8 ± 0.2	1.8 ± 0.3	1.8 ± 0.2	1.7 ± 0.12
Hepatosomatic index (HSI)	1.8 ± 0.2^{b}	1.6 ± 0.3^{b}	1.6 ± 0.2^{b}	1.8 ± 0.3^{b}	1.2 ± 0.2^{a}
Viscerosomatic index (VSI)	15.6 ± 3.6^{a}	16.0 ± 3.1^{a}	17.4 ± 2.7^{a}	22.7 ± 3.1^{b}	23.0 ± 3.0^{b}
Dress-out (DO)	84.4 ± 3.6^{b}	84.0 ± 3.0^{b}	82.6 ± 2.7^{b}	77.0 ± 3.0^{a}	77.3 ± 2.8^{a}

Dietary codes: *C* = control, RU5 = 5 % raw *Ulva* inclusion, RU10 = 10 % raw *Ulva* inclusion, AU5 = 5 % autoclaved *Ulva* inclusion, AU10 = 10 % autoclaved *Ulva* inclusion. $n = 9$

In the same line, values with different superscript letters are significantly different ($P < 0.05$). Data are expressed as mean ± SD

Table 6 Whole-body composition of rainbow trout fed the experimental diets at the end of 12-week feeding trial

%	Initial	*C*	RU5	RU10	AU5	AU10
Dry matter	25.4 ± 1.4	33.5 ± 1.1	32.2 ± 1.8	32.8 ± 2.0	29.9 ± 1.7	32.1 ± 2.1
Protein	12.6 ± 1.4	17.9 ± 0.9^{c}	17.9 ± 1.6^{c}	17.4 ± 1.3^{bc}	15.0 ± 1.2^{a}	16.6 ± 1.5^{b}
Lipid	9.1 ± 0.8	13.3 ± 0.7	12.2 ± 1.1	13.1 ± 1.4	12.4 ± 1.1	13.2 ± 1.4
Ash	3.6 ± 0.3	2.3 ± 0.4	2.2 ± 0.3	2.3 ± 0.6	2.3 ± 0.5	2.4 ± 0.6

Dietary codes: *C* = control, RU5 = 5 % raw *Ulva* inclusion, RU10 = 10 % raw *Ulva* inclusion, AU5 = 5 % autoclaved *Ulva* inclusion, AU10 = 10 % autoclaved *Ulva* inclusion. $n = 9$

In the same line, values with different superscript letters are significantly different ($P < 0.05$). Data are expressed as mean ± SD

Discussion

The results from the present trial indicate that the inclusion of raw *Ulva* meal at levels of up to 10 % in practical diets did not cause adverse effects on the growth performance, nutrient utilization, digestibility and muscle fatty acid composition of rainbow trout. Similarly, Valente et al. (2006) observed that 10 % dietary raw *Ulva* meal had no significant effect on growth performance of European sea bass and Güroy et al. (2007) reported that dietary *Ulva rigida* meal inclusion at 10 % had no negative effects on the growth of tilapia. Other studies have shown that a 5 % inclusion of dietary *Ulva* can have a positive influence on the growth performance of black sea bream *Acanthopagrus schlegeli* (Nakagawa et al. 1993) and snakehead *Channa striatus* (Hassan and Hashim 1995). In the present study, the inclusion of autoclaved *Ulva* meal, at both inclusion levels, led to significant reductions in body protein levels. This was not the case when using raw *Ulva* meal. No other body composition parameters (i.e., moisture, ash and lipid) were affected by the dietary treatments.

Many studies have sought to improve the digestibility and nutritive value of plant feedstuffs by thermal treatment methods (Barrows et al. 2007), and thus in the present study, autoclaved *Ulva* meal was studied in comparison with raw *Ulva* meal. The growth performance of fish fed diets containing 10 % autoclaved *Ulva* meal (group AU10) was significantly lower than that of fish fed 10 % raw *Ulva* meal (RU10); however, the performance was not significantly different to the control. To the authors' knowledge, there is no information regarding the effects of autoclaved *Ulva* meal with respect to growth performance of fish. However, many studies have assessed the effects of thermal

Table 7 Fatty acid composition (% of fatty acids) of rainbow trout fed the experimental diets at the end of 12-week feeding trial

	Initial	*C*	RU5	RU10	AU5	AU10
14:0	3.7 ± 0.1	6.4 ± 0.1	7.3 ± 0.1	7.3 ± 0.2	7.1 ± 0.2	7.2 ± 0.1
15:0	0.9 ± 0.0	1.0 ± 0.0	1.2 ± 0.1	1.1 ± 0.0	0.9 ± 0.0	1.0 ± 0.0
16:0	20.1 ± 0.3	25.1 ± 0.5	25.7 ± 0.4	25.7 ± 0.2	23.6 ± 0.1	24.9 ± 0.3
17:0	0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.7 ± 0.0	0.7 ± 0.0
18:0	6.9 ± 0.1	8.0 ± 0.2	7.1 ± 0.1	7.1 ± 0.1	7.0 ± 0.2	7.1 ± 0.1
20:0	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.1
∑Saturated[a]	35.2 ± 0.3	43.6 ± 0.4	43.9 ± 0.2	43.9 ± 0.1	40.6 ± 0.1	41.8 ± 0.3
18:1n-9	28.3 ± 0.1	22.2 ± 0.1	22.5 ± 0.2	22.5 ± 0.1	21.6 ± 0.1	21.8 ± 0.2
18:1n-7	2.3 ± 0.1	2.9 ± 0.3	3.9 ± 0.2	3.7 ± 0.1	2.9 ± 0.4	3.3 ± 0.1
20:1n-9	4.1 ± 0.2	3.8 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.5 ± 0.1	2.3 ± 0.2
∑Monounsaturated[b]	44.5 ± 0.1	39.3 ± 0.1	36.1 ± 0.2	34.0 ± 0.3	33.7 ± 0.1	33.9 ± 0.1
18:2n-6	3.9 ± 0.0	2.9 ± 0.0	2.9 ± 0.0	2.8 ± 0.0	2.6 ± 0.0	2.5 ± 0.0
18:3n-6	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
20:3n-6	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
20:4n-6	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
18:3n-3	3.3 ± 0.1	0.6 ± 0.2^{a}	0.9 ± 0.1^{ab}	0.9 ± 0.1^{ab}	1.2 ± 0.1^{b}	1.3 ± 0.1^{b}
18:4n-3	0.0 ± 0.0	0.07 ± 0.1^{a}	0.1 ± 0.1^{b}	0.2 ± 0.1^{b}	0.7 ± 0.1^{c}	0.7 ± 0.1^{c}
20:3n-3	0.9 ± 0.1	0.2 ± 0.1^{a}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	0.7 ± 0.2^{b}	0.7 ± 0.3^{b}
20:5n-3	3.1 ± 0.1	3.1 ± 0.2^{a}	3.8 ± 0.3^{ab}	4.5 ± 0.3^{b}	4.7 ± 0.3^{b}	4.0 ± 0.1^{b}
22:5n-3	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.2
22:6n-3	7.0 ± 0.2	9.1 ± 0.1^{a}	11.0 ± 0.3^{b}	12.0 ± 0.4^{b}	13.5 ± 0.3^{b}	12.9 ± 0.2^{b}
∑Polyunsaturated[c]	19.9 ± 0.1	17.0 ± 0.1^{a}	20.0 ± 0.1^{b}	21.6 ± 0.2^{b}	24.9 ± 0.4^{b}	24.3 ± 0.4^{b}
∑ n-6	4.3 ± 0.2	3.6 ± 0.1	3.7 ± 0.1	3.5 ± 0.4	3.4 ± 0.3	3.3 ± 0.1
∑ n-3	14.7 ± 0.1	14.5 ± 0.2^{a}	16.3 ± 0.1^{b}	17.7 ± 0.1^{b}	19.5 ± 0.2^{b}	18.3 ± 0.2^{b}
∑ n-3/n-6	3.4 ± 0.0	4.0 ± 0.1^{a}	4.4 ± 0.1^{b}	5.1 ± 0.0^{b}	5.7 ± 0.0^{b}	5.6 ± 0.1^{b}

Dietary codes: *C* = control, RU5 = 5 % raw *Ulva* inclusion, RU10 = 10 % raw *Ulva* inclusion, AU5 = 5 % autoclaved *Ulva* inclusion, AU10 = 10 % autoclaved *Ulva* inclusion. $n = 9$

In the same line, values with different superscript letters are significantly different ($P < 0.05$)

Data are expressed as mean ± SD

[a] Includes: 21:0, 22:0, 23:0, 24:0

[b] Includes: 14:1, 15:1, C16:1, C17:1, 22:1n-9

[c] Includes: 18:4n-3, 20:3n-6

processing on plant ingredients incorporated into aquafeeds (Satoh et al. 1998; Mwachireya et al. 1999; Allan and Booth 2004; Davies and Gouveia 2010). Thermal treatment applied throughout the commercial processing of plant products inactivates many of the anti-nutritional factors. The duration of the heat treatment should be kept to a minimum in order to minimize the possibility of destroying indispensable amino acids and vitamins, and reducing the availability of other nutrients (Peres et al. 2003).

The linolenic acid (18:3n-3) content of fish fed the AU5 and AU10 diets was significantly higher than control group. The sums of polyunsaturated fatty acid in the muscle of trout fed the *Ulva* diets (raw and autoclaved) were significantly superior when compared to control diet. Little information is available regarding muscle fatty acid composition of fish

fed dietary algae. However, Dantagnan et al. (2009) reported that the inclusion of 3 and 6 % macroalgae meal resulted in a significant increase in polyunsaturated fatty acids (PUFAs), especially EPA (20:5n-3), DHA (22:6n-3) and linolenic acid, in rainbow trout muscle, and Walker et al. (2009) observed that the arachidonic acid (20:4n-6) levels were elevated in Atlantic cod *Gadus morhua* juveniles fed *Porphyra* (30 % fishmeal replace).

The ADCs of dietary lipid were high, with no significant differences being observed between treatment groups. The ADCs for protein of all *Ulva* groups was significantly higher than the control group, and no differences between the *Ulva* treatment groups were observed. In contrast to these findings, previous studies have reported reduced ADCs for protein (Appler 1985) and lipid (Valente et al. 2006) in fish fed algal meals.

The present experiment indicates that raw *Ulva* meal has potential as a novel feed additive for inclusion in diets for rainbow trout juveniles. Dietary inclusion at levels up to 10 % can be used without detrimental effects on the growth performance, nutrient utilization, digestibility or muscle fatty acid composition. In fact, the inclusion of raw and autoclaved *Ulva* meal in rainbow trout diets improved the n-3 fatty acids content in rainbow trout muscle. Further studies are required to examine the effects on health and lipid metabolism, but it should be noted that high levels of autoclaved *Ulva* meal lead to poorer performance than raw *Ulva* meal.

Acknowledgments The authors kindly thank Zeki Sönmez, İzzet Şahin, Volkan Tepe and Serhan Mantoğlu for their technical assistance. We would also like to thank Agromey Feed Mill Company and Kartal Chemical Incorporated for providing feed ingredients.

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