Aquaculture Research

Aquaculture Research, 2009, **41**, 87–94 doi: 10.1111/j.1365-2109.2009.02308.x

Inclusion of macroalgae meal (*Macrocystis pyrifera*) as feed ingredient for rainbow trout (*Oncorhynchus mykiss*): effect on flesh fatty acid composition

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Abstract

The use of macroalgae as an additional component in animal feeding has been studied. However, information on how it could influence muscle composition of fish body is scarce. This study evaluates four diets with different macroalgae inclusion levels (0%, 1.5%, 3% and 6%) to test the effect on body fatty acid composition of rainbow trout. Tanks with a volume of 600 L were stocked with 60.6 ± 7.9 g fish at a density of 45 individuals tank^{-1} and fed for 124 days. At the end of the experiment there were not significant differences ($P < 0.05$) in muscle proximate composition among fish fed the different treatments. However, it was determined that inclusion of 3% and 6% of macroalgae meal resulted in a significant increase ($P < 0.05$) of polyunsaturated fatty acids (PUFAs) in muscle. In summary, macroalgae meal in rainbow trout diets do not enhance the quantity of protein and lipid contents at muscle level but an addition of 3–6% might contribute to increase the level of PUFAs, specially EPA, DHA and LIN. Thus, use of macroalgae meal might help to increase lipid quality content in the final product due the beneficial effects of PUFAs for human health.

Keywords: rainbow trout, *Macrocystis pyrifera*, aquafeed ingredients, fatty acids

Introduction

Available nutrients in aquafeeds have an important influence on various physiological functions of fish, such as reproduction, immune system and disease resistance (Satoh, Takeuchi & Watanabe 1987; Springate 1990; Carrillo & Zanuy 1995; Kiron, Fukuda, Takeuchi & Watanabe 1995; Koven, Barr, Lutzky, Ben-Atia, Weiss, Harel, Behrens & Tandler 2001). Furthermore, they can also influence the dietary excess of nitrogen and phosphorus loading to the environment, growth and surviving rate, digestibility and body composition (Watanabe 1982; Sargent, Henderson & Tocher 1989; Lall 1991; Gouveia, Teles, Gomes & Rema 1993). Composition of the final product is particularly important in aquaculture given that fish consumption has been recommended as an excellent source of nutrients that are scarce in terrestrial origin foods. In this sense, aquaculture nutrition plays an important role in the production of a valuable food for human.

Macroalgae has been often utilized as an ingredient in the livestock and poultry feed, raw or processed as meals, the last form being the most recommended due to the high mineral content (Waaland 1981). Marine plants are rich in a large variety of natural compounds and are used as a nutrient and/or in pharmaceutics. Marine macroalgae are promising sources of polyunsaturated fatty acids (PUFAs), which are extensively investigated for their favourable effects on human and animal health (Colombo, Risè, Giavarini, De Angelis, Galli & Bolis 2006). The usefulness of algae as a dietary ingredient for marine fish feeds has been investigated. Valente, Gouveia, Rema, Matos, Gomes and Pinto (2006) suggest that macroalgae such as *Gracilaria bursa-pastoris*, *Ulva*

rigida and *Gracilaria cornea* have great potential as alternative ingredients in diets for European sea bass juveniles at dietary inclusion levels up to 10% for *G. bursa-pastoris* and *U. rigida* and up to 5% inclusion level for *G. cornea* with no adverse effects on growth performance and feed utilization efficiency. A preliminary investigation conducted by Davies, Brown and Camilleri (1997) has demonstrated that while the macroalgae *Porphyra purpurea* is of lower nutritional value than conventional ingredients in semi-purified diets for the thick-lipped mullet (*Chelon labrosus*), partial substitution of fish meal with seaweed may prove to be cost effective. Nakagawa (1997) indicate that the use of macroalgae as a feed additive for fish accelerates the assimilation of ascorbic acid, improving the physiological conditions related to nutrition, of vitamin C and the metabolism of lipids altogether. Furthermore, it has been probed that the use of Kelp meal (*Macrocystis pyrifera*) in pelletized feeds for shrimps, works as an excellent additive (attractant, agglutinant and texturizer) that allow a more efficient utilization of dietary nutrients, reducing lixiviation and ensuring a better ingestion (Cruz-Suárez, Ricque-Marie, Tapia-Salazar & Guajardo-Barbosa 2000). Another study indicates that *Ascophylum* sp. meal appears to be a good source for inclusion as feed additive for red sea bream increasing the muscle protein in relation to the algae level added to the diet (Nakagawa 1997). Yet, research concerning the effect of using macroalgae meals in diets for fish is limited. The chemical composition of macroalgae can be different among species and highly determined by the geographical location and environmental conditions. From the nutritional point of view, the dehydrated macroalgae meal is an ingredient low in calories, with a high concentration of minerals and rich in carbohydrates, which are low digestible but that can contribute to the retention of water and minerals (Rodríguez & Hernández 1991; Castro, Carrillo & Perez 1994). However, in the case of lipid it does not surpasses a maximum of 1% (Jiménez & Goñi 1999). The contents of algae fatty acids could vary depending upon the species and the environmental conditions, such as temperature (Colombo *et al.* 2006).

One of the most important nutrients, and the reason fish consumption has been promoted around the world as a healthy food, are the lipids of high quality, specifically essential highly unsaturated fatty acids (HUFA), mainly eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA), which are more abundant in aquatic organisms and scarce in foods of terrestrial origin. The mechanisms of utilization and modification, deduced from the incorporation of these nutrients in the diet, as well as those originated by the biosynthesis processes, might be different and determined by the species, the developmental stage and environmental or physiological conditions. For this reason, studies aiming to incorporate new ingredients in fish diets must not attempt only to improve productive parameters, like growth and digestibility. It is important that these novel additives do not alter negatively the nutritional quality of the fish, like protein concentration in the body or the PUFA content principally the ω-3/ω-6 HUFA ratio and the levels of EPA and DHA, as well as the organoleptic characteristics (Thomassen & Rosjo 1989). The general objective of this study was to analyse the effect due to the inclusion of macroalgae meal in the diets for rainbow trout on flesh fatty acid composition.

Materials and methods

Experimental conditions

The experiment was conducted in 'Los Laureles' fish farming station belonging to the Aquaculture School, Catholic University of Temuco in Chile. Juveniles of rainbow trout with an approximate average body weight of 60.6 ± 7.9 g were randomly selected and distributed in 12 fibreglass tanks with a capacity of 600 L at a density of 45 fish tank^{-1}. Triplicate groups were assigned to each treatment and the feeding experiment was conducted for 124 days. The tanks were exposed to natural photoperiod and they had a continuous water supply with a renewal rate of 1.5 times h^{-1}. Average daily water temperature during the experimental period was 10.4 ± 1.2 °C. At the beginning of the experiment, fish were starved for 5 days before initiating the feeding with the experimental diets twice daily. Fish were given *ad libitum* access to food. Feed consumption, mortality and water quality parameters such as temperature and dissolved oxygen were monitored daily.

Experimental diets

Diets were prepared by a commercial feed company based in Chile using fishmeal (Super Prime, Exapesca S.A., Talcahuano, Chile), corn gluten meal (Graneles Chile S.A., Santiago, Chile) and hydrolysed feather meal (Terramar Chile S.A., Santiago, Chile) as main protein ingredients. Four diets were prepared and

Table 1 Proximate composition content of the diets employed in this study

	Control	T1	T2	T3
Proximate composition (%)*				
Dry matter	88.7 ± 0.1	93.1 ± 0.0	94.2 ± 0.0	91.8 ± 0.10
Protein	52.3 ± 0.1	53.2 ± 0.5	51.5 ± 0.1	52.8 ± 0.3
Lipid	22.9 ± 0.5	19.7 ± 0.2	23.1 ± 0.7	19.4 ± 0.6
Nitrogen free extractives	14.1 ± 0.7	16.3 ± 0.3	15.1 ± 0.6	16.4 ± 0.4
Fibre	1.3 ± 0.2	1.1 ± 0.5	1.0 ± 0.1	1.2 ± 0.1
Ash	9.4 ± 0.0	9.6 ± 0.0	9.3 ± 0.0	10.1 ± 0.0

*Values represent means ± SD ($n = 3$).

they were designated as control, T1, T2 and T3 respectively. The diet without addition of macroalgae meal was used as control. Diets T1, T2 and T3 were supplemented with 1.5%, 3.0% and 6.0% of macroalgae meal respectively. Proximal composition and fatty acid profiles of diets employed in the trial are shown in Tables 1 and 2 respectively.

Biochemical composition

Proximate and fatty acid profile in diets and muscle of fish were determined at the beginning and at end of the experiment to evaluate the effect of the different treatments. All analyses of samples were made in two replicates. Moisture was determined by following the Official Methods of Analysis of the Association of Official Analytical Chemists (Association of Official Analytical Chemists 1995). Total lipid was extracted from samples using a mix of chloroform:methanol (2:1) and its percentage (dry basis) determined as described by Folch, Less and Sloane-Stanley (1957). Lipid extracts were stored at −80 °C until further preparation for the analysis of fatty acid. Fatty acids were analysed by separation in a gas chromatograph (Hewlett Packard 5890 series II Plus, Wilmington, NC, USA) using a 30 m × 0.25 mm i.d. × 0.20 μm capillary column SP™ 2380 (Supelco, Bellafonte, PA, USA). Helium was used as a carrier gas. Fatty acids were analysed by comparison with a well-characterized standard such as SUPELCO™ 37 component FAME Mix (Sigma-Aldrich, St. Louis, MO, USA). Fatty acids were expressed in dry basis as per cent of the total fatty acids identified. Crude protein content was determined based on the total nitrogen composition (% protein = % N × 6.25) of the samples and through the Kjeldhal technique. Crude fibre was determined through acid digestion of previously defatted samples with H_2SO_4 followed by alkaline digestion with NaOH (0.313 N). The resulting residue was dried in an oven at 105 °C, weighed and incinerated at 550 °C for 30 min, finally the residue was weighed again and the proportion of fibre calculated. Crude ash content was determined by incinerating a known amount of the sample in an electric muffle furnace (Vulcan A550, NEY, Yucaipa, CA, USA) at 550 °C for 3 h.

Statistical analyses

Statistical analyses were performed using one-way analysis of variance with the software STATISTICA (StatSoft, Tulsa, OK, USA). Differences between treatments were studied using Tukey's test (Sokal & Rohlf 1980). Level of significance was set at $P < 0.05$.

Results

The results of growth performance and feed utilization are summarized in Table 3. Fish fed the experimental diets had growth responses almost comparable with that of the control group throughout the 124 days of feeding. Proximal composition at the end of the experiment revealed that the percentage of dry matter, fibre and ash did not differ significantly ($P > 0.05$) in the muscle of fish despite incorporation and concentration increment of macroalgae meal in the diets. However, protein and total lipids changed significantly ($P < 0.05$) in fish fed all diets (Table 4).

Fatty acid composition in muscle of rainbow trout fed different diets is shown in Table 5. It was found that with all diets the proportion of PUFAs and monounsaturated fatty acids increased in the muscle of fish at the end of the feeding experiment, representing in both cases around 30% of the total fatty acids, in comparison with that found in the initial group which was only 21.4% and 18.6% of the total respectively. Saturated fatty acid decreased from 60% in the initial sample of muscle to around 40% at the end of

Table 2 Fatty acid composition of the diets employed in this study*

Fatty acids (%)	Algal meal	Control	T1	T2	T3
SAFA					
12:0	0.000	0.012	0.013	0.010	0.011
13:0	0.001	0.011	0.010	0.010	0.011
14:0	0.084	0.940	0.871	0.817	0.837
15:0	0.012	0.126	0.122	0.114	0.119
16:0	0.305	3.198	3.150	2.832	2.950
17:0	0.011	0.218	0.225	0.204	0.198
18:0	0.101	0.873	0.882	0.799	0.833
20:0	0.000	0.058	0.062	0.054	0.091
21:0	0.000	0.009	0.054	0.047	0.027
23:0	0.000	0.029	0.030	0.032	0.007
24:0	0.004	0.011	0.016	0.020	0.010
Total SAFA	0.517	5.484	5.434	4.940	5.094
MUFA					
14:1	0.009	0.024	0.023	0.021	0.022
15:1	0.003	0.000	0.000	0.000	0.000
16:1	0.020	0.928	0.904	0.818	0.834
17:1	0.010	0.200	0.200	0.179	0.179
18:1ω9	0.242	3.136	3.100	3.052	2.930
20:1ω9	0.014	0.225	0.208	0.213	0.210
22:1ω9	0.000	0.000	0.000	0.000	0.000
24:1ω9	0.005	0.017	0.031	0.041	0.037
Total MUFA	0.304	4.530	4.465	4.323	4.210
PUFA					
18:2 ω 6t	0.006	0.296	0.299	0.265	0.269
18:2 ω 6c	0.042	1.301	1.179	1.244	1.316
18:3 ω 3	0.011	0.143	0.144	0.134	0.151
18:3 ω 6	0.003	0.024	0.032	0.027	0.023
20:2	0.003	0.037	0.042	0.040	0.036
20:3 ω 3	0.002	0.014	0.016	0.019	0.017
20:3 ω 6	0.002	0.011	0.011	0.013	0.014
20:4 ω 6	0.010	0.196	0.196	0.204	0.212
20:5 ω 3	0.077	1.663	1.532	1.528	1.423
22:6 ω 3	0.213	1.749	1.589	1.503	1.545
Total PUFA	0.369	5.435	5.041	4.977	5.005
$\sum$ ω-3 PUFA	0.303	3.57	3.28	3.18	3.14
$\sum$ ω-6 PUFA	0.066	1.83	1.72	1.75	1.83
ω-3/ω -6	4.84	1.95	1.91	1.82	1.71

*Dry matter basis.
SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

the feeding period. Moreover, it was found that for diets T2 and T3 the content of PUFAs in the muscle was much higher and significantly different ($P < 0.05$) in comparison with those found in control and T1 diets. Besides, it can be observed that the ratio of ω-3/ω-6 PUFA increased with all diets (around 2.40 and 2.83) in comparison with the initial ratio which was only 0.53. This is because with all diets there was a higher increase in the fatty acids from the series ω-3 than those from the series ω-6. In contras, it was found that the effect of diets over specific fatty acids, like EPA and DHA, in both cases increased in relation to the initial samples for all diets. In the case of EPA, the increment was from 0.29% to 2.58% and DHA raised from 0% to 5.6% for diet T3 with 6% macroalgae meal. Moreover, both fatty acids increased significantly ($P < 0.05$) for diets T2 and T3 in comparison with control and T1 diets.

Discussion

The proximate analyses of the diets reveal that addition of macroalgae meal does not affect the level of

Table 3 Growth performance of rainbow trout fed experimental diets with different macroalgae meal contents

	Control	T1	T2	T3
Initial body weight (g)	60.60 ± 7.9	60.60 ± 7.9	60.60 ± 7.9	60.60 ± 7.9
Final body weight (g)	351.71 ± 24.08	341.28 ± 15.99	331.61 ± 18.19	331.80 ± 13.21
FCR	1.05 ± 0.09	1.05 ± 0.11	1.01 ± 0.13	1.04 ± 0.12
SGR	1.42 ± 0.05	1.39 ± 0.03	1.37 ± 0.05	1.37 ± 0.03

FCR, feed conversion ratio; SGR, specific growth rate.

Table 4 Proximate composition content in the flesh of rainbow trout fed diets containing different levels of macroalgae meal

	Initial	Control	T1	T2	T3
Proximate composition (%)*					
Dry matter	17.02	11.44 ± 1.87	10.77 ± 2.42	8.29 ± 1.18	11.29 ± 3.15
Protein	80.17	71.33 ± 2.39^a	71.60 ± 0.18^a	70.41 ± 1.76^a	66.35 ± 1.17^b
Lipid	12.2	22.79 ± 2.40^a	22.07 ± 0.24^a	22.57 ± 1.61^a	27.82 ± 1.04^b
Nitrogen-free extractives	1.61	0.28 ± 0.14^a	0.80 ± 0.07^b	1.65 ± 0.00^c	0.54 ± 0.03^a
Fibre	0.47	0.38 ± 0.17	0.26 ± 0.04	0.34 ± 0.14	0.42 ± 0.16
Ash	5.55	5.22 ± 0.33	5.28 ± 0.32	5.04 ± 0.01	4.9 ± 0.04

*Values with different letter within a line are significantly different ($P < 0.05$).
Values represent means ± SD ($n = 3$).

fibre and lipids among them regardless the percentage of inclusion. Nevertheless, the levels of lipids in the all experimental diets are within the requirements recommended for rainbow trout (Guillaume, Kaushik, Bergot & Métailler 2001). Only a supplementation of 6% macroalgae meal in diet T3 makes a significant increment in the total amount of ash present in the feed. Cruz-Suárez *et al.* (2000) found that with the inclusion of 8% kelp meal, content of ash and fibre increased significantly with respect to food control from 6.6% to 9.3% and 0.99% to 1.4% which agree with the results obtained in this investigation. The same authors establish for shrimp that kelp meal increases the capacity of water absorption of food across its phycocoloid compounds, generating an activity of agglutination that facilitates consumption of food without generating many crumbs and enabling better utilization of nutrients. However, in dry matter, the addition of macroalgae meal contributed to reduce the percentage of water in the feed that might represent an advantage in relation to the stability of the pellet in the water column. Therefore, further research is required to evaluate the incorporation of macroalgae meal on the agglutination and texturization of the pellet, an aspect that was not considered in this experiment.

The main sites of lipids storage in the fish are the liver, visceral fat and muscle; of these, only the muscle is actually suitable for human consumption. As indicated previously muscle of fish fed diet T3 with the higher inclusion of macroalgae meal presented a significantly increased amount of lipids ($P < 0.05$). These results are in agreement with those of other authors who have studied the nutritional value of different seaweeds (*Undaria penatifida* and *Ascophyllum nodosum*) as dietary supplement for red sea bream (*Pagrus major*) (Yone, Furuichi & Urano 1986; Nakagawa, Umino & Tasaka 1997). These studies suggest that seaweed contributes to the lipid deposition to the muscle and mobilize the lipid to energy (Yone *et al.* 1986).

The fatty acid composition of the four experimental diets is similar, furthermore there are no significant quantitative differences related to the addition or lack of the macroalgae meal. This demonstrates that the nutritional contribution of fatty acid from the macroalgae meal is scarce or nil. Moreover, the balance of ω-3 and ω-6 fatty acids series, EPA and DHA do not vary in relation to the control diet.

All diets, including the control (without macroalgae meal addition), have levels of highly polyunsaturated fatty acids over 3%, with an important content of EPA and DHA, even higher than what is recommended for freshwater fish (Sargent, Bell, Bell, Henderson & Tocher 1995).

The absence of dietary long chain ω-3 and ω-6 fatty acids could be replaced by the incorporation of short chain ω-3 and ω-6 fatty acids into the diet because

Table 5 Fatty acid composition in the flesh of rainbow trout fed diets employed in this study (% dry matter basis)*

Fatty acids	Initial	Control	T1	T2	T3
SAFA					
12:0	0.05	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.02
13:0	0.00	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01
14:0	0.56	1.13 ± 0.42^{a}	1.22 ± 0.35^{a}	2.46 ± 0.42^{b}	2.13 ± 0.82^{b}
15:0	0.05	0.18 ± 0.07^{a}	0.19 ± 0.04^{a}	0.36 ± 0.05^{b}	0.32 ± 0.13^{ab}
16:0	1.25	6.04 ± 2.33	6.54 ± 1.39	11.78 ± 1.85	10.42 ± 4.13
17:0	0.52	0.26 ± 0.14^{a}	0.20 ± 0.03^{a}	0.39 ± 0.08^{b}	0.35 ± 0.14^{ab}
18:0	0.47	1.37 ± 0.50^{a}	1.43 ± 0.28^{a}	2.40 ± 0.40^{b}	2.06 ± 0.93^{ab}
20:0	0.16	0.09 ± 0.03^{a}	0.05 ± 0.05^{a}	0.15 ± 0.03^{b}	0.14 ± 0.05^{b}
21:0	0.00	0.08 ± 0.05^{a}	0.04 ± 0.03^{a}	0.15 ± 0.08^{ab}	0.19 ± 0.06^{b}
23:0	0.00	0.20 ± 0.09^{a}	0.25 ± 0.11^{a}	0.36 ± 0.02^{b}	0.32 ± 0.10^{b}
24:0	0.00	0.08 ± 0.03	0.29 ± 0.35	0.13 ± 0.06	0.11 ± 0.04
Total SAFA	3.06	9.45 ± 3.66^{a}	10.27 ± 1.87^{a}	18.23 ± 2.98^{b}	16.08 ± 6.41^{b}
MUFA					
14:1	0.03	0.05 ± 0.03^{a}	0.05 ± 0.02^{a}	0.12 ± 0.02^{b}	0.11 ± 0.04^{b}
15:1	0.01	0.03 ± 0.01^{a}	0.04 ± 0.01^{a}	0.08 ± 0.01^{b}	0.07 ± 0.03^{ab}
16:1	0.07	0.41 ± 0.53^{a}	1.36 ± 0.45^{b}	2.92 ± 0.63^{c}	2.55 ± 0.94^{c}
17:1	0.02	0.13 ± 0.07	0.19 ± 0.06	0.30 ± 0.10	0.35 ± 0.16
18:1 ω 9	0.74	4.82 ± 1.58^{a}	4.82 ± 1.43^{a}	9.33 ± 1.93^{b}	8.36 ± 3.29^{b}
20:1 ω 9	0.01	0.29 ± 0.11^{a}	0.25 ± 0.07^{a}	0.53 ± 0.10^{b}	0.44 ± 0.23^{ab}
22:1 ω 9	0.00	0.02 ± 0.01	0.05 ± 0.03	0.05 ± 0.01	0.03 ± 0.01
24:1 ω 9	0.02	0.11 ± 0.04^{a}	0.20 ± 0.13^{b}	0.18 ± 0.10^{b}	0.13 ± 0.03^{a}
Total MUFA	0.93	6.59 ± 1.93^{a}	7.85 ± 2.10^{a}	15.14 ± 2.62^{b}	13.51 ± 5.31^{b}
PUFA					
18:2 ω 6t	0.00	0.11 ± 0.03^{a}	0.11 ± 0.04^{a}	0.24 ± 0.05^{b}	0.22 ± 0.06^{b}
18:2 ω 6c	0.18	1.58 ± 0.60^{a}	1.55 ± 0.45^{a}	3.11 ± 0.48^{b}	2.88 ± 1.05^{b}
18:3 ω 3	0.03	0.18 ± 0.06^{a}	0.17 ± 0.05^{a}	0.33 ± 0.05^{b}	0.31 ± 0.11^{b}
18:3 ω 6	0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.02
20:2	0.11	0.07 ± 0.05^{a}	0.10 ± 0.03^{ab}	0.15 ± 0.04^{b}	0.15 ± 0.08^{b}
20:3 ω 3	0.02	0.04 ± 0.02^{a}	0.05 ± 0.01^{a}	0.09 ± 0.02^{b}	0.07 ± 0.03^{ab}
20:3 ω 6	0.01	0.02 ± 0.00	0.07 ± 0.08	0.01 ± 0.01	0.01 ± 0.01
20:4 ω 6	0.42	0.26 ± 0.10^{a}	0.29 ± 0.06^{a}	0.47 ± 0.06^{b}	0.41 ± 0.16^{b}
20:5 ω 3	0.29	1.66 ± 0.72^{a}	1.42 ± 0.51^{a}	2.96 ± 0.65^{b}	2.58 ± 1.00^{b}
22:6 ω 3	0.00	3.77 ± 1.54^{a}	4.01 ± 0.87^{a}	5.95 ± 0.81^{b}	5.62 ± 2.30^{b}
Total PUFA	1.07	7.73 ± 3.12^{a}	7.84 ± 1.23^{a}	13.36 ± 2.12^{b}	12.30 ± 4.75^{b}
Ó ω-3 PUFA	0.33	5.66 ± 2.34^{a}	5.65 ± 0.85^{a}	9.33 ± 1.48^{b}	8.58 ± 3.39^{b}
Ó ω-6 PUFA	0.62	1.99 ± 0.74^{a}	2.04 ± 0.48^{a}	3.88 ± 0.60^{b}	3.57 ± 1.30^{b}
ω-3/ω -6	0.53	2.83 ± 0.29	2.76 ± 0.30	2.41 ± 0.06	2.40 ± 0.29

*Values with different letter within a line are significantly different ($P < 0.05$).
Values represent means ± SD ($n = 3$).
SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

freshwater fish have the ability to desaturate fatty acids. The incorporation of only ω-3 fatty acids is not enough; there must be a balance between both types of fatty acids in the diet in order to meet the requirements (Watanabe 1982; Sargent *et al.* 1995). In this sense, all experimental diets maintain a ratio of ω-3/ω-6 close to 2, which is within the characteristic requirements for freshwater fish in general.

It has been recognized that diets rich in lipids increase the lipid deposition in the body of fish and in the same way the nature of the lipids in the diet have a great influence on the fatty acid composition of the body. For example, in rainbow trout fed with diets containing significant amounts of corn oil it is possible to find high concentrations of ω-6 in the muscle, while when the diet is formulated with fish oil the levels of ω-3 increases (Guillaume *et al.* 2001). Even when the fatty acid content in the tested diet was similar, the effect on the fatty acid composition of the fish muscle was different, resulting in a higher increment of the PUFAs, specifically EPA, DHA and linoleic acid in those fish fed the diets T2 and T3. Studies

conducted with laying hens suggest that the incorporation of 10% *M. pyrifera* in the diets is an effective way to increase the ω-3 fatty acid content (Carrillo, López, Casas, Avila, Castillo, Carranco, Calvo & Pérez-gil 2008). Thus, it can be inferred that there may exist a synergic effect from specific unknown components present in the macroalgae meal that were not considered in this experiment and that might affect the lipid metabolism in fish. Nakagawa(1997) reported that the effect of dietary algae on the improvement of lipid metabolism in fish can be partially explained using the synergistic effect with vitamin C, which is an important component of most brown algae, like *M. pyrifera* used in this experiment (Cruz-Suárez *et al.* 2000). Further research is required to elucidate the macroalgae meal use on aquafeeds.

In conclusion, it is possible to deduce that the inclusion of $> 3\%$ macroalgae meal in diets for rainbow trout might provide a promising means to enhance lipid utilization and the quality of muscle in fish. The main contribution of this promising ingredient could be to improve the levels of EPA and DHA that have been indicated as important and beneficial factors for human nutrition and health (Steffens 1997). Thus, inclusion of macroalgae meal represents a way to increase the nutritional value of farmed freshwater fish as a source of essential fatty acid for humans.

Acknowledgment

This study has been financed by CONICYT (Consejo Nacional de Ciencia y Tecnología) in the frame of the project FONDEF D01I1164.

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