Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
21(2): 262-267, Mar./Apr. 2011

Using *Macrocystis pyrifera* (L.) C. Agardh from southern Chile as a source of applied biological compounds

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Article

Received 8 Jan 2011
Accepted 28 Jan 2011
Available online 6 May 2011

Keywords:
aquaculture
kelps
Macrocystis pyrifera
Magellan region *Oncorhynchus mykiss*

ISSN 0102-695X
doi: 10.1590/S0102-695X2011005000072

Abstract: The exploitation of seaweeds in Chile has been carried out for more than 60 years. More recently, seaweeds have been used for the production of alginate, agar and carrageenan, agricultural fertilizers and industrial aquaculture (feed for abalone and sea urchins), increasing the added value of this natural resource. In the Magellan Region (56°S), the giant kelp *Macrocystis pyrifera* (L.) C. Agardh presents the most extensive kelp forest, reaching a biomass of approximately 12 $kg.m^{-2}$. Recent studies have shown potential benefits from adding *M. pyrifera*-derived flour to salmonid feed. Research is currently underway to evaluate the use of brown algae-derived products for marine aquaculture feed of *Oncorhynchus mykiss* in tanks. There was no apparent adverse effect on the evaluated parameters that can be attributed to the incorporation of *M. pyrifera* meal in the diets fed to salmonids. Even when the control diet had numerically the best performance in zootechnical terms, the analysis of variance of all parameters evaluated showed no significant differences with regard to diets containing *M. pyrifera* meal. These results demonstrated that seaweed meal has important benefits for animal health and nutrition that could be applied or tested in other marine organisms of commercial importance.

Introduction

Some of the world´s most extensive kelp forests are exclusive to the southernmost Chilean Patagonia (Magellan Strait 53-54°S), which include species of *Lessonia, Durvillaea antarctica* and notable dominance of *Macrocystis pyrifera*. *M. pyrifera* is distributed along the Chilean coast from Iquique to Cape Horn (Palacios & Mansilla, 2003; Plana et al., 2007). The populations of *M. pyrifera* are distributed parallel to the coast, forming continuous and irregular seaweed beds 30 to 45 m wide. The subtidal kelp forest grows down to 15 m, providing habitat, refuge, food and breeding sites for other marine flora and fauna (Castilla & Durán, 1985; Avila et al., 1982). *M. pyrifera* is among the largest seaweeds found in the Magellan Region, reaching a size up to 90 m in length.

The commercial importance of *M. pyrifera* in Chile has increased over the last decade, especially for extraction of phycocolloids such as alginates, for food additive and dietary supplement production for human consumption and animal feed, as a bio-stimulant for agricultural crops, and for the manufacture of pharmaceuticals and cosmetics, among others (Mansilla et al., 2009).

The presence of essential aminoacids and fatty acids in *M. pyrifera* has recently been reported and its high quality proteins and lipids are comparable to those of some terrestrial plant species (Cruz et al., 2000; Palacios & Mansilla, 2003). It may therefore provide important dietary supplements for human consumption, for bird feed, and as a plant bio-stimulant, as well as a food supplement for the salmon farming industry (Mansilla et al., 2005). Nutritionally, algae of the genus *Macrocystis* provide a low-calorie low-fat food source, high in minerals (Mg, Ca, P, K and I), vitamins, protein, non-highly digestible carbohydrates, and fiber (Jiménez-Escrig & Goñi-Cambrodon, 1999). Chemical compounds of algae vary considerably, depending on factors such as the species, geographical distribution and habitat (*e.g.*, depth, wave exposure), season (*e.g.*, temperature), developmental stage, influence of oceanic currents and nutrient concentrations in the environment, among others.

The aim of the present study was to evaluate the use of *Macrocystis pyrifera*-derived flour as a dietary supplement for intensive salmonid culturing.

Materials and Methods

Experimental design of cultures

We used twelve tanks of 1000 L, maintained with continuous aeration at an average water temperature of 13 to 15 ºC. We placed 45 rainbow trout (*Oncorhynchus mykiss*) with an average individual weight of 63 g each per tank. The trout were maintained for five days in acclimation processes and subsequently fed *ad libitum*. Periodic measurements of fish weight and length were made to evaluate the daily weight and length growth rate for each treatment. The samples included 10% of the fish in each tank.

Preparation of diets

The diets were prepared in a food processing facility using extrusion processes and following procedures commonly used in the salmon industry (AOAC, 1995). Four experimental diets were used in the experiments: i. commercial feed, used as a control (D1); ii. commercial feed with 1.5% seaweed meal (D2); iii. commercial feed with 3.0% seaweed meal (D3) and iv. commercial feed with 6.0% seaweed meal (D4). Each diet was tested in three trials. We used the FORMAT software (calculation program based on linear programming) for feed formulation, considering the following general restrictions, which take into account salmonid nutritional requirements by age and species.

To evaluate the digestive use of feed, one kilogram of each of the four diet types was ground and sieved (300 µm mesh) and 1.0% Cr_2O_3 was added as inert marker and mixed for 40 min in a Kitchenaid model K5SS mixer. The mixture was moistened with 30% water and pelleted in a RCA 1HP with a matrix of 3.5 mm holes. The wet pellets were oven dried for 24 h at 65 °C. Subsequently, the experimental diets were stored in bags and refrigerated at -20 °C until they were used.

Seasonal nutritional profile of macroalgal flour

Seaweed meal processed from the extraction in all four seasons (summer, autumn, winter and spring) was analyzed by proximate analysis, amino acid profile, fatty acids and minerals.

Digestibility of raw materials

To assess the digestibility of the raw material (flour of *M. pyrifera*), a modification of the method developed by Bureau et al. (1999) was employed. The digestibility of raw material of two diets consisting of 80-20% and 70-30% reference diet and seaweed meal, respectively was determined. For the preparation of each test diet, the methodology described above under Preparation of Diets was employed.

Statistical treatments

We used one-way analysis of variance (ANOVA), followed by comparisons of the means with Tukey´s test (95% confidence level). Prior to the implementation of the ANOVA, we applied the homogeneity of variance test and the arc cosine transformation, where $f(x) = Cos^{-1} \sqrt{(1-X)}$ is described by Sokal & Rohlf (1969) for percentage data. All statistical analyses were done with the Statmost 3.0 software program.

Results

Seasonal nutritional profile of macroalgal flour

The seasonal analysis of *Macrocystis* flour showed that the protein and ash contents are higher during spring, lipids and fibers in summer and carbohydrates in winter time (Table 1).

Table 1. Seasonal analysis of *Macrocystis pyrifera* flour.

Season	Protein %	Lipids %	Carbohydrates %	Fibers %	Ash %
Spring	17.48	0.40	3.27	20.02	37.18
Summer	10.24	0.84	5.26	20.43	31.92
Autumm	9.18	0.61	6.12	14.58	35.57
Winter	11.0	0.47	8.46	18.94	29.88

Chemical composition of diets

The chemical composition of the control and the experimental diets prepared with seaweed meal did not show significant differences in protein, fibers, ash or nitrogen-free compounds. Significant differences were observed in the dry matter and the ethereal extracts (Table 2).

Coefficient of apparent digestibility of raw material

Total CAD (Coefficient of Aapparent Digestibility) ranged between 85.98% (diet 4) and 86.38% (diet 1), but were not significantly different ($p>0.05$). CAD values showed significant differences ($p<0.05$) (94.20% control diet and 94.65% diet 4). We found no statistically significant differences ($p>0.05$) between the ethereal extract or the nitrogen-free extract for the CAD. Finally, the lowest CAD of energy

Table 2. Chemical composition of the diets used in the bioassay of digestibility.

	Diet 1 Control	Sd	Diet 2 1.5%	sd	Diet 3 3.0%	sd	Diet 4 6.0%	sd
% Dry Matter	96.35[a]	0.05	96.07[b]	0.04	96.09[b]	0.00	96.79[cd]	0.03
% Protein	51.84[a]	0.32	50.86[a]	1.87	49.01[a]	2.21	52.16[a]	0.27
% Ethereal extract	23.95[a]	0.09	22.97[b]	0.17	23.46[a]	0.04	19.46[c]	0.14
% E. Nitrogen-free*	13.40[a]	0.33	14.75[a]	1.31	16.83[a]	2.14	16.80[a]	0.42
% Fiber	0.77[a]	0.09	0.89[a]	0.19	0.80[a]	0.05	0.98[a]	0.06
% Ashes	10.04[a]	0.01	10.53[a]	0.54	9.90[a]	0.16	10.60[a]	0.05
% Cr_2O_3	1.07	0.03	1.00	0.05	1.10	0.03	1.09	0.00

[a,b,c,d] Values in the same row with different superscripts are significantly different (p<0.05); The values are the average with a standard deviation (sd) (n=2 replicates); *Nitrogen-free extract; Calculated by difference (100-Total amount of other ingredients).

Table 3. Percentage of aminoacids in the diets used in the bioassay of digestibility (% aa/100g diet).

	Diet 1 Control	Diet 2 1.5%	Diet 3 3.0%	Diet 4 6.0%
Asp	3.2	2.3	1.7	3.3
Glu	5.7	4.7	4.6	5.7
Ser	3.8	3.7	1.6	1.5
Gly	0.8	1.5	3.5	2.9
His	6.4	6.1	7.0	5.7
Arg	3.3	2.3	3.0	3.1
Thr	2.1	2.8	2.3	1.9
Ala	3.1	4.8	2.7	2.7
Pro	3.1	4.9	4.2	2.6
Tyr	1.0	1.0	1.2	1.0
Val	2.5	3.3	2.9	2.4
Met	0.9	1.0	1.1	1.1
Cys	1.0	1.0	1.1	1.0
Ileu	2.0	2.2	2.3	2.0
Leu	3.4	3.8	4.0	3.5
Phe	2.0	1.6	2.6	4.6
Lys	3.2	3.3	2.6	3.9
Met+Cys	1.9	2.0	2.2	2.1
Phe+Tyr	3.0	2.6	3.7	5.6

Table 4. Percentage of minerals in the diets used in the bioassay of digestibility.

	Diet 1 Control	Diet 2 1.5%	Diet 3 3.0%	Diet 4 6.0%
P	1.40	1.37	1.27	1.38
K	0.63	0.80	0.80	0.97
Ca	2.00	1.97	1.83	1.92
Mg	0.14	0.14	0.13	0.14
Na	0.57	0.58	0.55	0.61
Cu	0.000983	0.000995	0.001380	0.0019300
Zn	0.0204	0.0217	0.0207	0.0193
Mn	0.0109	0.0121	0.0114	0.0107
Fe	0.0171	0.0212	0.0233	0.0278
B	0.00113	0.00108	0.00117	0.00153

content was observed for diet 4 (93.49%), which was significantly lower (p<0.05) than those of the other three diets (Table 5).

Composition of feces

Table 6 shows the approximate composition of feces collected during the bioassay of the digestibility of raw material. The analysis of the feces showed significant differences between dry matter and ashes

Table 5. Coefficient of apparent digestibility of the experimental diets.

	Diet 1 Control	Sd	Diet 2 1.5%	sd	Diet 3 3.0%	sd	Diet 4 6.0%	sd
Total	86.38[a]	0.65	86.33[a]	1.38	86.04[a]	0.43	85.98[a]	0.15
Protein	94.65[a]	0.20	94.61[a]	0.27	94.40a[b]	0.37	94.20[b]	0.19
Ethereal extract	99.21[a]	0.12	99.05[a]	0.19	99.17[a]	0.15	99.00[a]	0.03
E. nitrogen-free	72.57[a]	1.39	73.74[a]	3.12	74.55[a]	2.08	74.94[a]	0.18
Energy	94.32[a]	0.27	94.08[a]	0.52	94.21[a]	0.20	93.49[b]	0.08

[a,b,c,d] Values in the same row with different superscripts are significantly different (p<0.05). The values are the average with a standard deviation (sd) (n=2 replicates).

Table 6. Composition of feces collected in the test of the digestibility.

	Diet 1 Control	Sd	Diet 2 1.5%	sd	Diet 3 3.0%	sd	Diet 4 6.0%	sd
% Dry matter	21.56[a]	1.20	20.76[b]	0.13	19.43[c]	0.60	19.01[d]	0.48
% Protein	20.37[a]	0.26	20.13[ab]	1.03	19.66[b]	0.98	21.56[c]	0.53
% Ethereal extract	1.39[a]	0.16	1.59[a]	0.22	1.40[a]	0.29	1.39[a]	0.05
% E. Nitrogen-free*	26.99[a]	0.41	28.28[a]	1.06	30.69[bc]	2.48	30.03[c]	0.40
% Fiber	5.11[a]	0.25	5.74[b]	0.05	6.26[c]	0.09	6.10[cd]	0.59
% Ashes	46.14[a]	0.10	44.26[b]	1.10	41.99[c]	1.27	40.92[d]	0.14
% Cr_2O_3	7.52	0.38	7.64	0.71	7.63	0.24	7.85	0.09

[a,b,c,d] Values in the same row with different superscripts are significantly different (p<0.05). The values are the average with a standard deviation (sd) (n=2 replicates); *Nitrogen-free extract; Calculated by difference (100 - Total amount of other ingredients).

between all of the diets (Table 6). The percentage of protein was significantly higher in diet 4 than in diet 2 (Table 6). There were no significant differences in the ethereal extract between the diets. The lowest values of the nitrogen-free extract and of fiber in the feces were in the control diet, with 27% and 5.11%, respectively; on the contrary, diet 3 had the highest proportions of these, with 30.68% and 6.26%, respectively.

Digestibility of minerals

Overall, no significant differences were observed in the digestibility of minerals at the level of inclusion of the algae in the diets. There were no significant differences (p>0.05) for magnesium, sodium, copper, zinc and manganese between the diets. Phosphorous content also showed no significant differences between diets 1 and 4 (Table 6).

Diet 4 potassium content was significantly (p<0.05) higher than that in the other three diets. Diet 3 had the lowest value of the CAD for Calcium (13.23%). There were differences in the CAD (p<0.05) between diets 1 and 4 for boron content, but not between diet 1 and the control.

There were minor variations in the mineral content between the diets (Table 7) and small differences in the aminoacid contents (aspartic acid, serine, glycine, phenylalanine, proline, and alanine content; Table 8). No statistical comparisons were possible since only a single application of the treatment was performed.

Survival

The survival rate showed that the treatments have an influence during the first 34 days of culture, in which diet 3 showed the greatest effects with 88% survival, followed by diets 2 and 3 with 96% survival. Over the next 68 days, all treatments were stable, continuing to fall slightly to 103 days. For the entire 124 day experimental period, the the control treatment finished with better overall survival (94%) followed

Table 7. Percentage of minerals in feces collected in the bioassay of digestibility.

	Diet 1 Control	Diet 2 1.5%	Diet 3 3.0%	Diet 4 6.0%
P	4.92	4.82	4.35	4.17
K	0.02	0.02	0.02	0.02
Ca	11.03	11.63	11.37	10.70
Mg	0.33	0.32	0.29	0.31
Na	2.52	2.52	2.15	2.11
Cu	0.00287	0.00275	0.00295	0.00334
Zn	0.0948	0.1019	0.0959	0.0862
Mn	0.0653	0.0698	0.0662	0.0581
Fe	0.0653	0.0698	0.0662	0.0581
B	0.00411	0.00452	0.00405	0.00473

Table 8. Percentage of aminoacids in feces collected in the bioassay of digestibility (% aa/100g feces).

	Diet 1 Control	Diet 2 1.5%	Diet 3 3.0%	Diet 4 6.0%
Asp	0.6	0.3	1.3	0.5
Glu	1.1	0.6	0.9	1.2
Ser	1.4	1.1	1.4	1.7
Gly	0.4	0.2	0.2	0.3
His	2.9	2.2	2.8	3.3
Arg	0.8	0.7	0.7	0.6
Thr	1.4	0.8	1.0	1.0
Ala	2.9	1.1	1.4	1.5
Pro	3.5	1.6	1.8	1.7
Tyr	0.6	0.7	0.6	0.5
Val	1.7	1.1	1.4	1.7
Met	1.7	0.9	0.1	0.6
Cys	0.4	0.5	0.7	0.7
Ileu	0.7	0.9	1.0	1.2
Leu	1.1	1.5	1.7	2.1
Phe	0.7	1.0	0.9	1.4
Lys	1.1	1.3	1.4	1.4
Met+Cys	2.2	1.5	0.8	1.4
Phe+Tyr	1.3	1.6	1.5	1.8

by diet 4 (90%) and diet 2 (89%) and finally diet 3 (85%). Although the best overall survival was obtained with the control diet, it should be noted that, from day 38 to 124, diet 3 showed the highest survival rate. The analysis of variance indicated that there were no significant differences between the treatments (p=0.405) or between the means.

In the work of Bureau et al. (2003), a survival of 98% was obtained, while in this work survival between 85 and 94% was achieved, in particular 90% for diet 4 with 6% algae. The two variables, individual weight and survival, are the important ones in terms of net biomass gain.

Total biomass growth

The increase in total biomass obtained in the first 68 days with each diet showed no major noticeable differences, although the biomass of the control diet was slightly higher at the end of the experiment, finishing with 14.9 kg. For the period from 103 to 124 days, the total biomasses were in the descending order diet 1, diet 4 and diet 3 with values of 13.63, 13.49 and 12.75 kg, respectively. No significant differences were found in the analysis of variance (p=0.322) or the Tukey test for means.

There were no apparent adverse effects on the evaluated parameters that could be attributed to the incorporation of *Macrocystis pyrifera* meal into the diets administered to salmonids. Even though the control diet had the best zootechnical performance in numerical terms, the analysis of variance of all the parameters evaluated showed that there were no statistically significant differences relative to the diets containing *M. pyrifera* meal.

Digestibility

The incorporation of 1.5, 3.0, or 6.0% of algal meal into the diet did not affect the total digestibility, the digestibility of lipids or carbohydrate digestibility. However, the diet with 6% algal meal decreased the digestibility of protein and energy.

There was no evidence that the digestibility of minerals is affected by the incorporation of algae into the diets. However, there was a tendency to increase the digestibility of phosphorus in the diet with an addition of 6% algae. Although not conclusive, this effect appears to be interesting from the environmental point of view.

In contrast, high levels of inclusion (20 and 30%) of *M. pyrifera* algal meal produce decreases in the coefficients of total digestibility of protein, lipids, and energy; however, there was no decrease in the coefficient of apparent digestibility (CAD) of the nitrogen-free extract.

As a raw material, the digestibility of algae meal is low, demonstrating that this ingredient can only be used in small amounts in the diet of fish grown commercially.

Conclusions

The incorporation of seaweed meal, at the level employed in the three experimental diets, did not affect the total digestibility of lipids or carbohydrates. The digestibility of minerals was apparently not affected. However, there was a tendency to increase the digestibility of phosphorus in diet 4 (addition of 6% seaweed meal), an important result in the context of environmental sustainability. The digestibility of algal meal as a raw material is low, demonstrating that this ingredient should only be used in small amounts in fish diets in commercial fish farms. The greatest nutritional value of seaweed meal is represented by the nitrogen-free extract and the ashes, proteins and lipids being very low in the meal. Consequently, seaweed meal cannot be considered as a real substitute for proteins or lipids, but rather as a supplement for minerals and carbohydrates. The inclusion of seaweed meal does not qualitatively or quantitatively improve the level of protein or lipids in the diet, given the profile of proteins, lipids, fatty acids and aminoacids; however, the contribution of minerals is significant with 6% inclusion of seaweed meal in the diet. The effect of inclusion of seaweed meal in the diets on the ω3/ω6 ratio is almost negligible, but the percentage of polyunsaturated fatty acids in the muscle (mainly EPA, DHA and linoleic acid) increased when the level of inclusion was 3 or 6%. The health of the fish during the experiment was optimal. Histopathological examination of various fish organs fed with diets of algae showed no morphological or functional alterations that would compromise their homeostatic response

These results demonstrate that seaweed meal has important benefits for animal health and nutrition that could be applied or tested in other marine organisms of commercial importance.

Acknowledgment

The authors thank the support of PFB-23 (Basal-CONICYT), P05-002 (ICM-MIDEPLAN), Project DO1I1164 Fondef - Conicyt. The technical assistance of Aliro Borquez in the bioassays is greatly appreciated. S. Murcia, C. Anderson and E. Velasquez collaborated in the translation and revision of the manuscript in English.

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