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Marine microalgae from biorefinery as a potential feed protein source for Atlantic salmon, common carp and whiteleg shrimp

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Abstract

Two marine algal products MAP3 and MAP8 were examined for their suitability as fishmeal protein substitutes in feeds of three prominent farmed species, through short-term feeding studies. Algal meals were tested at 5 and 10% protein replacement levels for Atlantic salmon and at 25 and 40% for common carp and whiteleg shrimp. At the end of the 12-week period, the growth and feed performance of the two fish species did not reveal any significant difference between those fish offered the algae-based feed and those offered the control feed. The whole body proximate compositions of Atlantic salmon fed the control and algae-based feeds were not significantly different. In common carp, the lipid content in the fish fed higher level of MAP3 was significantly lower than that of the fish fed the control feed. In whiteleg shrimp, at the end of the 9-week feeding period, growth performance and feed utilization did not differ between the treatment groups. Protein content in the shrimp fed the higher level of MAP8 was significantly lower than that of shrimp on the control feed. The three species could accept the algal meals in their feeds at the tested levels, though there were some noticeable effects on body composition at higher inclusion levels.

KEY WORDS: aquatic feeds, Atlantic salmon, common carp, microalgae, protein source, whiteleg shrimp

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Introduction

In recent years, the aquaculture industry has succeeded in reducing the inclusion rates of fishmeal and fish oil in the feeds of farmed aquatic animals. However, due to the increase in production of all farmed species there is still a growing demand for these ingredients (Naylor *et al.* 2009). Fishmeal is the principal source of protein in commercial aquatic feeds. As a result of the steep increase in price of fishmeal and the decline in fishery resources that go into the fishmeal production, there is an interest in developing alternatives to this finite component. Finding and testing alternate protein and lipid sources is important to the aquatic feed industry.

As microalgae protein is of good quality, with amino acid profiles comparable to that of other reference food proteins (Becker 2007), it could be a plausible alternative to fishmeal protein. In addition, microalgae, which are the source of all photosynthetically fixed carbon in the food web of aquatic animals (Hardy 1924; Kwak & Zedler 1997), may be an ideal replacement for fishmeal in aquatic feeds. Meal from the cyanobacterium *Spirulina*, a brackish-water genus that is neither a eukaryote nor marine, has been incorporated into experimental fish feeds with some success (El-Sayed 1994; Olvera-Novoa *et al.* 1998; Nandeesha *et al.* 2001; Palmegiano *et al.* 2005). However, there have been few investigations of the use of marine microalgae in compound aquatic feeds (e.g. Jaime-Ceballos *et al.* 2006), especially not on microalgae produced in large-scale

quantities. Microalgal co-products resulting from the production of third generation biofuels, which may be available in large amounts in the future are a source of human food and animal feed protein (Brennan & Owende 2010; Stephens *et al*. 2010). Mass produced microalgae are therefore a promising ingredient in aquafeeds.

Determining the suitability of the available algae co-product is as challenging as finding replacement for fishmeal in aquafeeds. We investigated whether commercially produced marine microalgae (one species in the form of a co-product of biofuel production and a second species used as whole algae without the biorefining process) could replace fishmeal in the feeds of three important, and substantially different species farmed widely in world aquaculture. Atlantic salmon (*Salmo salar* L.) require a sizable portion of fishmeal in their feeds (Tacon & Metian 2008), while whiteleg shrimp (*Litopenaeus vannamei* Boone) can tolerate feeds devoid of fishmeal (Amaya *et al*. 2007b). Common carp (*Cyprinus carpio* L.) typically utilize a smaller proportion of fishmeal; however, since carp production volumes are large, the total usage of fishmeal is high (Tacon & Metian 2008). The objectives of the short-term feeding trials described here were to determine if the inclusion of selected algae, in the feeds of the chosen species, could affect their growth and carcass composition.

Materials and methods

Design of the studies

Three separate short-term pilot feeding studies were designed to test two different microalgal products as potential replacements for fishmeal protein in feeds of Atlantic salmon, common carp and whiteleg shrimp. Four algae-based feeds (two types of alga at two levels) were tested against a control fishmeal feed in order to: (i) determine their ability to produce growth comparable to the control feed and (ii) identify any significant changes in body composition. The experiment on salmon was conducted in Norway and those on carp and shrimp were performed in Thailand. The studies are presented species-wise in the different sections.

Algal products

The two algae used in this study are novel isolates of the genera *Nanofrustulum* (Bacillariophyceae) and *Tetraselmis* (Chlorophyceae) from coastal waters of the Pacific Ocean surrounding Hawaii, referred to forthwith as MAP3 and MAP8, respectively. Both strains were isolated in 2008 and are being cultured on a commercial scale as sources of biofuel. These strains were cultivated in November and December 2008 at the facilities of Cellana (Cellana LLC, Kona, Hawaii) in a two-stage cultivation system that comprised of photobioreactors and ponds with unit capacities of 25 and 60 m^3, respectively (Huntley & Redalje 2007). Photobioreactor cultures were partially harvested each day to inoculate pond batch cultures, which, after attaining maximum production, were harvested and ultimately dewatered by centrifugation. The centrifuged product was then dried (about 60 °C) to <3% moisture in a spray dryer, and stored at 4 °C until further processing. This production process was repeated for several days for each strain until we accumulated approximately 1000 kg dry weight of whole algae meal.

Algae meals MAP8 (oil not extracted), and MAP3 (oil extracted) were used for the growth and feeding trials. Defatted meal, MAP3 was prepared by POS Pilot Plant Corporation (Saskatchewan, Canada) using a hexane extraction to separate the neutral lipid fraction, then evaporating the residual hexane from the remaining defatted biomass - a process similar to the separation of soy oil from soya beans, which also yields a defatted biomass suitable for animal consumption. Both products are hereafter referred to as algal meal; their proximate compositions are provided in Table 1. The two meals are relatively rich in polar lipids, especially MAP3, from which neutral lipids were preferentially extracted. The ash of MAP3 contained a significant amount of silica, characteristic of diatoms.

Atlantic salmon

Experimental set-up and fish The feeding experiment on Atlantic salmon was conducted in an indoor fish rearing facility at the Research Station, University of Nordland, Bodø, Norway. The rearing unit consisted of 520-L fiber glass tanks (approx. 450-L water volume), each having a flow-through system supplying seawater at a rate of 0.5 L sec^{-1} from a depth of 250 m in Saltfjorden. The water temperature in the tanks was 8 °C and the dissolved oxygen saturation was maintained above 90% during the rearing period. The fish husbandry followed the practices approved by the State Authorities who periodically examined the set-up. Lighting conditions were arranged so as to gradually illuminate the rearing hall from 06:00 to 22:00 (16:8 day/night). The study was approved by the Norwegian Animal Research Authority (Mattilsynet, Forsøksdyrutvalget, Brumunddal, Norway).

Aqua Gen strain (Aqua Gen AS, Sluppen, Trondheim, Norway) Atlantic salmon post-smolts (62 g; 0-year) were

Table 1 Composition of the two microalgal products

	MAP3	MAP8
Proximate composition[1]		
Moisture	31.5	108.0
Protein	118.9	278.6
Lipid	31.4	38.0
Ash	530.7	201.3
Essential amino acid composition[2,3]		
Histidine	1.13	1.18
Threonine	5.08	5.24
Arginine	6.22	5.60
Valine	4.91	5.23
Methionine	2.42	2.25
Isoleucine	4.05	4.03
Tryptophan	1.12	1.05
Phenylalanine	4.51	4.77
Leucine	6.79	7.50
Lysine	7.21	6.67
Composition of major fatty acids[3,4]		
C14:0	6.58	4.29
C16:0	25.97	24.70
C16:1n7	38.31	26.15
C18:1n11	1.80	7.85
C18:2n6	3.09	7.50
C18:3n3	0.66	4.99
C20:4n6	4.42	3.82
C20:5n3	9.53	7.93
ΣSaturates	33.5	30.1
ΣMUFA	42.8	37.1
ΣPUFA	23.1	29.3

[1] dry weight, g kg^{-1}.
[2] g 100 g^{-1} protein.
[3] Kiron *et al.* (unpublished data).
[4] g 100 g^{-1} total fatty acid.

purchased from Mainstream Norway (Bunes Division, Bodø, Norway) and maintained at the Research Station, University of Nordland for a period of five months on commercial feeds until they were used for the trial. At the start of the feeding trial the fish had an average weight of 173.1 g. Fish were sorted and allotted randomly to each feed group. Twenty-five fish were introduced into each of the 15 tanks (triplicate tanks per treatment), achieving an average density of 9.7 kg m^{-3}.

Feeds and feeding regime A control fishmeal-based feed and four algae-based feeds were formulated. The two algae products (MAP3 referred to by the suffix 3 and MAP8 referred to by the suffix 8 in the feed codes) replaced 5 and 10% of the fishmeal protein in the respective experimental feeds. The feeds are referred to as CO for the control; L3, H3 for alga MAP3 at 5 and 10% replacement levels, respectively; and L8 and H8 for alga MAP8 at 5 and 10% replacement levels, respectively. The ingredient and proximate composition of the feeds are given in Table 2. To prepare the respective feeds, the ingredients mentioned in the table were first mixed thoroughly in a mixer (Varimixer Bear RN 20 VL2, A/S Wodschow, Broendby, Denmark). The dough was then passed through a mincer (Sirman TC22 RIO, Sirman SpA, Curtarolo, Italy) to prepare the pellets, which were then dried at 35 °C for 20 h (Rational SCC 101, Rational AG, Landsberga, Germany). Pellets were graded to a size 4–5 mm, vacuum packed and stored at 4 °C. The amounts required for a week's use were kept in containers at 4 °C for daily feeding.

Atlantic salmon were fed manually six times weekly with the experimental feeds: once daily from 07:30 to 11:00 (in three cycles to satiety). The hand feeding regime was chosen to observe the appetite of the fish. Apparent feed intake was recorded daily during the entire 12-week period.

Performance The individual fish weights from each tank were recorded at the start, at the 6th week (to monitor the growth of fish) and at the end of the feeding period (end of 12th week). The fish were fasted for 48 h prior to sampling. When fish were removed due to death or other reasons, their respective weights were recorded. Growth and feed performance were assessed for each 6-week segment as well as for the entire 12-week period. The following formulae were used to calculate the growth performance parameters: Survival (%) = 100 $[N_f/N_i]$, where N_f is the final fish number and N_i is the initial fish number; Weight gain (%) = $[(W_f - W_i)/W_i] \times 100$, where W_i and W_f represent the initial body weight and final body weight; Specific growth rate, SGR (% day^{-1}) = $100 \times [(\ln W_f - \ln W_i)/D]$, where W_i and W_f represent initial and final weights (tank means, g), respectively, and D represents the number of feeding days.

To determine the body composition at the start, nine fish were collected and minced together to form the initial sample. At the 12th week, three fish each were collected from replicate tanks of each of the five feed groups and minced together in order to determine their respective body compositions. The moisture (gravimetry), ash (incineration at 550 °C), crude protein (N*6.25, Kjeldahl Autoanalyser, Tecator, Sweden) and lipid content (Bligh & Dyer 1959) of these samples, as well as those of feeds were determined.

Using the growth and the feed consumption data, the nutritive quality of the experimental feeds was calculated employing the following formulae: Daily apparent feed intake of the fish (% body weight day^{-1}) = $FI/W_m \times 100$ where $W_m = \sqrt{(W_f \times W_i)}$; W_i and W_f represent initial and final weights (tank means, g), respectively and FI is the average apparent feed intake (g) per fish per day. Feed conversion ratio, FCR = $F/(B_f\text{-}B_i\text{+}B_d)$, where F is the dry

Table 2 Composition of the experimental feeds for Atlantic salmon

Ingredients (g kg^{-1})	CO	L3	H3	L8	H8
Fishmeal[1]	280	266	252	266	252
MAP3	0	87	174	0	0
MAP8	0	0	0	37	74
Calanus meal[2]	319	319	319	319	319
Cellulose[3]	150	77	4	127	104
Suprex wheat[4]	150	150	150	150	150
Fish oil[5]	87	87	87	87	87
Mineral mix[6]	4	4	4	4	4
Vitamin mix[7]	10	10	10	10	10
Proximate composition[8] (g kg^{-1} dry weight)					
Moisture	38.9 ± 2.2	36.6 ± 5.8	42.9 ± 6.8	37.3 ± 3.9	43.0 ± 5.3
Crude protein	427.1 ± 10.5	429.5 ± 6.9	424.8 ± 4.2	429.1 ± 9.1	434.1 ± 15.1
Crude lipid	177.9 ± 4.7	180.7 ± 3.6	181.3 ± 2.3	179.4 ± 4.5	183.3 ± 17.1
Ash	91.0 ± 0.9	144.3 ± 1.6	190.3 ± 1.4	98.2 ± 0.5	107.9 ± 5.9
Gross energy (MJ kg^{-1})	21.8 ± 0.6	21.7 ± 0.5	21.8 ± 0.3	21.0 ± 0.1	19.96 ± 0.1

[1] Bodø Sildoljefabrikk AS, Bodø, Norway.
[2] Calanus AS, Tromsø, Norway.
[3] Sigma-Aldrich, Steinheim, Germany.
[4] Codrico, Rotterdam, The Netherlands.
[5] Bodø Sildoljefabrikk AS, Bodø, Norway.
[6] Mineral mix (g kg^{-1}): $MgSO_4$ – 2.477, KH_2PO_4 – 1.008, $ZnSO_4$ – 0.220, $FeSO_4$ – 0.249, $MnSO_4$ – 0.031, $CuSO_4$ – 0.013, $CoCl_2$ – 0.002, Na_2SeO_4 – 0.0012.
[7] Proprietary formulation of Skretting Aquaculture Research Center, Stavanger, Norway.
[8] Analyses of three batches of feed given as mean ± SD.

apparent feed intake (g), B_f is the final biomass (g), B_i is the intial biomass (g) and B_d is the biomass of dead fish (g); Protein efficiency ratio, PER = $(B_f\text{-}B_i)/PI$,where B_f is the final biomass (g), B_i is the intial biomass (g) and PI the protein intake (g).

Common carp

Experimental set-up and fish The feeding experiment on common carp was conducted at the indoor fish rearing facility of the Department of Aquatic Science, Prince of Songkla University, Songkhla, Thailand. The fish rearing units were 180-L glass aquaria (approx. 160-L water volume) that were aerated continuously. The average water temperature of the rearing tanks was 27 °C. The dissolved oxygen levels were monitored routinely during the rearing period.

Common carp fingerlings were obtained from Kamtan Farm, Hat Yai, Thailand and maintained at the facility for a period of two months on commercial feeds, prior to the start of the feeding trial. At the start, the fish having an average weight of 10.92 g were sorted and allotted randomly to a particular feed group. Twenty fish were introduced into each of the 15 tanks (triplicate tanks per treatment) in the set-up.

Feeds and feeding regime The microalgal products replaced 25 and 40%, respectively of the fishmeal protein in the experimental feeds. The feeds are referred to as CO for the control; L3, H3 for alga MAP3 at 25 and 40% replacement levels, respectively; and L8 and H8 for alga MAP8 at 25 and 40% replacement levels, respectively. The ingredients and proximate composition of feeds are given in Table 3. The analytical methods were same as those adopted for Atlantic salmon.

Three batches of the above feeds were prepared in the feed laboratory of Prince of Songkla University. The ingredients for these feeds were first mixed properly and then the dough was pelletized (Hobart A200T, Hobart Manufacturing Co., OH, USA). The pellets were then dried at 60 °C for 24 h (Memmert D06061, Memmert GmbH, Schwabach, Germany).The storage and handling of the pellets (size 2–3 mm) were similar to those mentioned for salmon feeds. The fish were fed manually everyday, twice daily between 08:00 and 16:00, to satiety. The apparent intake of the experimental feeds was recorded every two weeks and the cumulative consumption for the 12-week trial period was calculated.

Performance Individual fish weights from each tank were recorded at the start and at the end of the feeding period (end of 12th week). In addition, the total biomass from each tank was recorded at the 6th week to monitor the growth of the fish. Fish were fasted for 24 h prior

Table 3 Composition of the experimental feeds for common carp

Ingredients (g kg^{-1})	CO	L3	H3	L8	H8
Fishmeal[1]	160	120	96.1	120	96.1
MAP3	0	201.5	323	0	0
MAP8	0	0	0	106.9	171.2
Soybean meal[1]	370	370	370	370	370
Poultry meal[2]	62	68	70	65	65
Cassava[1]	264	99.5	2	196	156.5
Wheat gluten[1]	50	50	50	50	50
Fish oil[1]	37	34	32	35.1	34.2
Vitamin mixture[3]	10	10	10	10	10
Choline chloride[4]	6	6	6	6	6
Mineral mixture[5]	30	30	30	30	30
MSP[6]	11	11	11	11	11
Proximate composition[7] (g kg^{-1} dry weight)					
Moisture	47.0 ± 3.7	44.2 ± 1.2	46.9 ± 0.4	41.0 ± 0.8	41.0 ± 0.9
Crude protein	351.6 ± 0.2	354.7 ± 0.1	358.7 ± 0.8	354.9 ± 0.3	357.2 ± 0.6
Crude lipid	62.6 ± 2.4	63.8 ± 1.3	65.4 ± 1.6	69.9 ± 2.7	65.9 ± 4.5
Ash	138.4 ± 4.2	214.6 ± 1.8	253.3 ± 0.8	116.5 ± 2.7	126.4 ± 0.8
Gross energy (MJ kg^{-1})	19.5 ± 0.5	17.1 ± 0.1	16.0 ± 0.1	18.9 ± 0.2	18.8 ± 0.2

[1] CP Foods Public Co., Ltd., Songkhla, Thailand.
[2] Inteqc Feed Co., Ltd., Samutsakhon, Thailand.
[3] Vitamin mixture (mg kg^{-1} feed): Thiamine (B_1) 10; Riboflavin (B_2) 20; Pyridoxine (B_6) 10; Cobalamine (B_{12}) 2; Retinal (A) 4000 IU; Cholecaciferol (D_3) 2,000 IU; Menadione sodium bisulfate (K_3) 80; Folic acid 5; Calcium pantothenate 40; Inositol 400; Niacin 150; Tocopherol (E) 50; Biotin 3; Ascorbic acid (C) 500.
[4] DSM Nutritional Product, Samutprakan, Thailand.
[5] Mineral mixture (mg kg^{-1} feed): Na 98; Mg 758; K 2298; Ca 1473; Fe 145; Zn 20; Mn 13; Cu 2.07; Co 0.59; I 0.45.
[6] Ajax Finechem Pty Ltd., Australia.
[7] Analyses of 3 batches of feed given as mean ± SD.

to the mentioned sampling points. Mortality was also recorded.

To determine the body composition at the start, 10 fish were collected and minced together to form the initial sample. At the 12th week, three fish each were collected from replicate tanks of the five treatments in order to measure their carcass composition. The methods adopted were similar to those described for salmon, except that lipid was determined using soxhlet extraction apparatus (Soxtec System HT, Foss Tecator AB, Hoganas, Sweden) with methylene chloride as the extracting solvent. The performance parameters were calculated as described for Atlantic salmon.

Whiteleg shrimp

Experimental set-up and shrimp The feeding experiment on whiteleg shrimp was conducted at the indoor facility of the Aquatic Science Research Station, Prince of Songkla University, Songkhla, Thailand. Glass aquaria of size 235-L (water volume 200-L) used for rearing the shrimp were part of a continuously aerated flow-through system. The water temperature in the aquaria was 29 °C. Dissolved oxygen level and ammonia concentration were monitored regularly.

Whiteleg shrimp at postlarval stage 15 were obtained from Charoen Pokphand Hatchery, Songkhla, Thailand and maintained on a commercial feed in the experimental rearing units for three months before the start of feeding trials. The average body weight of the shrimp at the start was 2.21 g; 30 shrimp were randomly introduced into each of the 25 tanks (5 tanks per treatment) in the set-up.

Feeds and feeding regime In the experimental feeds, MAP3 and MAP8 were included to replace 25 and 40% of fishmeal protein – similar to that described for carp. The ingredients and proximate composition of feeds (CO, L3, H3, L8 and H8) are presented in Table 4. Three batches of each of the above feeds were prepared in the laboratory as dry pellets of size 2 mm, following the same procedure employed for carp feed. The feeds were stored and used as described for Atlantic salmon.

The shrimps were fed manually everyday, four times a day at 08:00, 12:00, 16:00 and 20:00, to satiety. The apparent intake of the experimental feeds was recorded everyday during the 9-week trial by visual inspection of the aquaria.

Table 4 Composition of the experimental feeds for whiteleg shrimp

Ingredients (g kg^{-1})	CO	L3	H3	L8	H8
Fishmeal[1]	155.2	116.3	93.1	116.3	93.1
MAP3	0	222.3	355.1	0	0
MAP8	0	0	0	106.4	170.3
Soybean meal[1]	211.2	211.2	211.2	211.2	211.2
Poultry meal[2]	155	155	155	155.1	155
Tapioca flour[1]	331.9	148.5	38.9	262.4	221.7
Wheat four[1]	30	30	30	30	30
Squid meal[1]	20	20	20	20	20
Probiotic[3]	10	10	10	10	10
Wheat gluten[1]	33.2	33.2	33.2	33.2	33.2
Fish oil[1]	10	10	10	12	12
Lecithin[1]	10	10	10	10	10
Cholesterol[1]	1.5	1.5	1.5	1.5	1.5
Vitamin premix & mineral premix[4]	25	25	25	25	25
Vitamin E[5]	0.8	0.8	0.8	0.8	0.8
Vitamin C[5]	1	1	1	1	1
BHT[6]	0.2	0.2	0.2	0.2	0.2
Zeolite[1]	5	5	5	5	5
Proximate composition[7] (g kg^{-1} dry weight)					
Moisture	38.2 ± 2.6	36.7 ± 0.5	38.2 ± 6.6	40.7 ± 2.1	48.6 ± 0.3
Crude protein	340.6 ± 0.3	355.8 ± 5.2	360.7 ± 0.2	352.8 ± 2.4	355.1 ± 0.5
Crude lipid	59 ± 1.7	85.4 ± 0.7	92.4 ± 2.4	93.3 ± 0.1	82.5 ± 2.2
Ash	85.3 ± 2.3	194.2 ± 4.7	250 ± 0.9	92.8 ± 3.7	93.9 ± 0.5
Gross energy (MJ kg^{-1})	19.3 ± 0.3	17.7 ± 0.9	15.9 ± 0.3	19.7 ± 0.2	19.8 ± 0.1

[1] CP Foods Public Co., Ltd., Songkhla, Thailand.
[2] Inteqc Feed Co., Ltd., Samutsakhon, Thailand.
[3] Kidchakan Supamattaya, Aquatic Animal Health Center, PSU, Songkhla, Thailand.
[4] Vitamin mix (mg kg^{-1} feed): Cyanocobalamin 0.096; Niacin 80; Riboflavin 60; Pantothenic acid 180; Menadione 40; Folic acid 6; Biotin 0.60; Thiamin 40; Pyridoxine 60; Inositol 400; Vitamin A 6000 IU; Vitamin D3 2000 IU; Vitamin E 250 IU. Mineral mix (mg kg^{-1} feed): Zn 72; Fe 36; Mn 12; Cu 24; Co 0.6; I 1.2; Cr 0.8; Se 0.2; Mo 0.2.
[5] DSM Nutritional Product, Samutprakan, Thailand.
[6] Sigma-Aldrich, Steinheim, Germany.
[7] Analyses of 3 batches of feed given as mean ± SD.

Performance The bulk weights of shrimp were recorded from each tank at the start of the feeding period, at the 4th week and at the end of the study (end of 9th week) in order to estimate the weight gain.

The initial body composition was determined from a minced sample obtained from 30 shrimps. At the 9th week, three shrimp were collected from each replicate of the five treatment tanks in order to analyse body composition, as described for common carp. The performance parameters were calculated as described in the earlier sections.

Statistical analysis

All observations from replicate tanks were analysed statistically using Graphpad Prism 5 (Graphpad Software Inc., La Jolla, CA, USA). The data were checked for normality (D'Agostino & Pearson omnibus normality test) and equal variance. Tukey's multiple comparison tests were used to check the differences between the groups. For non-parametric data, the Kruskal–Wallis test was used followed by Dunn's multiple comparison tests. The differences between groups were considered significant at $P < 0.05$.

Results and discussion

Recently there has been great interest in commercially grown microalgae, not only for biofuels, but also for products that could be processed for food, feed, pharmaceuticals and other high-value chemicals. We explored the possibility of using two microalgal meals, obtained as co-products from biorefinery, in the feeds of different aquatic species. Although the algal varieties varied in their proximate composition, their amino acid and fatty acid profiles (Table 1) suggest that they could be made into valuable ingredients for aquatic animal feeds. Based on the reported amino acid requirements of the species studied (Wilson 2002) the algal products will be able to provide most of the essential amino acids. The amounts of histidine, methionine

and phenylalanine in the algae will not be adequate for the three species. In the current research, we have only examined if the aquatic species can accommodate the algal meals in their feeds. As pointed out by Glencross *et al*. (2007) ingredient digestibility and palatability are also essential information in evaluating alternate ingredients in aquafeeds. Detailed investigations, covering these and other aspects will be undertaken in the next phase of this research.

Atlantic salmon

Growth performance, feed performance and body composition of salmon fed the algae-based feeds and those fed the control feed were not different ($P > 0.05$) (Tables 5 & 6).

The experimental feeds prepared by replacing a portion of the fishmeal protein with algal protein contained cellulose as the inert filler. Cellulose at levels up to 150 g kg^{-1} in the feeds of salmonids did not have any influence on the digestibility of the main nutrients (Aslaksen *et al*. 2007; Hansen & Storebakken 2007).

The inclusion of the algae did not reveal any statistically significant difference in the growth data. Alga MAP8 at 5% produced a weight gain of 62.6% during the 12-week period as against 61.6% for the control fish. With respect to SGR, FCR and PER, both algal products yielded similar performance values as that of the control feed. At the end of the trial, SGR values ranged from 0.48 to 0.58. The FCR values we observed (1.12–1.25) are comparable to published ranges for salmon during the spring season (0.58 –1.18; Einen *et al*. 2007). Protein efficiency ratio of the fish fed the low levels of algae (L3 = 2.08 and L8 = 2.09) was close to that of the control group (2.06), indicating that the replacement with algal protein may not have affected the

Table 5 Survival, growth and feed utilization during a 12-week feeding trial on Atlantic salmon offered microalgae-based feeds

	CO	L3	H3	L8	H8
Survival (%)	98.7 ± 2.22	100 ± 0.00	100 ± 0.00	100 ± 0.00	98.7 ± 2.22
Weight (g)					
Week 0	169.7 ± 22.2	173.2 ± 19.7	173.4 ± 16.4	168.6 ± 18.0	178 ± 20.8
Week 6	227.6 ± 18.0	229.9 ± 24.2	224.9 ± 20.1	230.5 ± 27.4	230.8 ± 25.3
Week 12	271.8 ± 42.7	271.7 ± 33.9	257.3 ± 34.7	272.4 ± 45.7	267.5 ± 43.8
Weight gain (%)	61.57 ± 6.95	56.09 ± 0.47	49.24 ± 4.33	62.59 ± 3.66	51.10 ± 1.24
SGR (% day^{-1})[1]	0.57 ± 0.05	0.53 ± 0.01	0.48 ± 0.03	0.58 ± 0.03	0.49 ± 0.01
FI (% BW day^{-1})[2]	0.74 ± 0.06	0.70 ± 0.02	0.70 ± 0.05	0.76 ± 0.03	0.67 ± 0.03
FCR[3]	1.14 ± 0.06	1.12 ± 0.03	1.25 ± 0.02	1.12 ± 0.01	1.17 ± 0.04
PER[4]	2.06 ± 0.11	2.08 ± 0.05	1.87 ± 0.04	2.09 ± 0.01	1.88 ± 0.17

Values are given as mean ± SD; $n = 3$ replicate tanks.
[1] Specific growth rate – SGR.
[2] Apparent feed intake – FI, Body weight – BW.
[3] Feed conversion ratio – FCR.
[4] Protein efficiency ratio – PER.

Table 6 Proximate composition of the whole fish and fillet from Atlantic salmon offered microalgae-based feeds for 12 weeks

	CO	L3	H3	L8	H8
Whole fish					
Moisture	723.7 ± 4.7	721.7 ± 8.1	721.7 ± 1.6	721.0 ± 3.7	721.6 ± 4.6
Lipid	270.4 ± 13.4	270.9 ± 10.5	296.1 ± 5.5	297.9 ± 8.7	292.7 ± 5.2
Protein	666.1 ± 18.8	668.9 ± 23.0	672.9 ± 12.9	660.4 ± 19.9	671.8 ± 12.4
Ash	86.2 ± 6.5	80.5 ± 5.6	83.9 ± 1.1	81.2 ± 5.8	82.5 ± 8.7
Fillet					
Moisture	753.9 ± 5.6	756.5 ± 3.9	757.4 ± 0.7	754.6 ± 4.7	760 ± 0.7
Lipid	126.3 ± 12.4[a]	100.6 ± 6.3	97.9 ± 2.8	96.1 ± 12.9	85.8 ± 5.9[b]
Protein	859.8 ± 13.9	870.3 ± 2.2	867.4 ± 6.0	864.5 ± 17.2	883.4 ± 10.3
Ash	61.9 ± 4.3	64.2 ± 7.7	69.5 ± 4.5	66.3 ± 1.4	62.6 ± 5.5

Values (g kg^{-1} dry weight) are given as mean ± SD; $n = 3$ fish from each of the three replicate tanks.
Different superscripts indicate statistically significant differences ($P < 0.05$), if any, between the groups in a particular row.

rate of protein utilization. However, both algae at the higher inclusion levels seemed to lower the PER values, though the effect was not statistically significant.

With the exception of reduced lipid content in H8 fillets (Table 6), other biochemical components assayed in the whole fish and fillet did not show any significant difference. The lipid content of the fillet was highest for the control group and this was significantly different ($P < 0.05$) from that recorded for the H8 group. A lipid lowering effect of algae diets has been observed previously. *Chlorella* extract was found to reduce lipid accumulation in the muscle of ayu, *Plecoglossus altivelis* (Nematipour *et al*. 1987). Similarly, dairy cows on a diet supplemented with the microalga, *Schizochytrium* sp., produced milk with lower fat content (Boeckaert *et al*. 2008). Furthermore, the inclusion of algal phospholipids would be of added advantage in aquatic feeds as phospholipids are known to improve the performance of teleost fish (Tocher *et al*. 2008).

No significant differences in growth or feed performance were observed for algae-based feeds relative to controls, at either the 5% replacement level or at the 10% replacement level. Atlantic salmon, being carnivorous, may not be capable of tolerating high amounts of plant materials in their feed (Krogdahl *et al*. 2003; Torstensen *et al*. 2008). Protein rich microalgae may be incorporated in salmon feeds at higher amounts than those tested in the present study. However, in a recent study on another carnivorous fish, Atlantic cod, replacement of 15 and 30% of fishmeal protein with a microalgae mix of *Nannochloropsis* sp. and *Isochrysis* sp. caused a significant growth reduction at the higher replacement level (Walker & Berlinsky 2011). It should be noted that the algae mix had an overall protein content of 420 g kg^{-1} and a lipid content of 180 g kg^{-1}, much higher than that of the algal meals used in the present study.

Common carp

The performance parameters of the groups of common carp that received algae-based feeds did not differ significantly from those of fish that were offered the control fishmeal-based feed (Table 7). Algal protein was used to replace fishmeal in the experimental feeds and cassava was incorporated to balance the nutrient composition. The inclusion of cassava up to 45% in the feeds of carp fingerlings was found to enhance both the carbohydrate and protein digestion (Ufodike & Matty 1983).

Common carp are omnivorous and can digest substantial amounts of carbohydrate from plants and may utilize the energy from this component more effectively than carnivorous fishes. In the present study, this ability is reflected in the growth rates attained by carp receiving algae at higher levels even though not statistically supported.

Other varieties of algae have been employed in feeding trials on carp, but direct comparisons are not attempted here because there are wide differences in biochemical profile between the algae. Atack *et al*. (1979) reported poor feed conversion for fingerling mirror carp (*C. carpio*) fed cyanobacterial protein (*Spirulina maxima*) compared to casein- or petroyeast-protein feeds of similar protein and energy values (2.50 vs. 1.39 for casein and 1.55 for petroyeast). The digestibility of the algae was also lower (87.1%) compared to casein (93%) and petroyeast (96.6%). The protein efficiency ratio of the algal feed was 1.15 as against 2.08 for petroyeast and 2.48 for casein. In another study

Table 7 Survival, growth and feed utilization during a 12-week feeding trial on common carp offered microalgae-based feeds

	CO	L3	H3	L8	H8
Survival (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	96.67 ± 5.77
Weight (g)					
Week 0	10.97 ± 0.02	10.93 ± 0.08	10.92 ± 0.07	10.93 ± 0.05	10.96 ± 0.03
Week 6	29.96 ± 2.51	30.55 ± 2.20	31.76 ± 0.14	31.85 ± 0.23	31.81 ± 2.87
Week 12	74.08 ± 6.85	69.18 ± 7.32	73.97 ± 0.52	74.53 ± 4.05	80.60 ± 10.29
Weight gain (%)	575.20 ± 61.34	532.90 ± 62.77	577.20 ± 8.32	582.00 ± 34.05	635.20 ± 91.85
SGR (% day^{-1})[1]	2.27 ± 0.11	2.19 ± 0.12	2.28 ± 0.01	2.28 ± 0.06	2.37 ± 0.15
FI (% of BWday^{-1})[2]	3.85 ± 0.19	4.26 ± 0.17	4.33 ± 0.10	4.04 ± 0.14	4.03 ± 0.19
FCR[3]	1.46 ± 0.06	1.70 ± 0.05	1.64 ± 0.03	1.52 ± 0.06	1.43 ± 0.09
PER[4]	1.95 ± 0.09	1.66 ± 0.05	1.70 ± 0.03	1.85 ± 0.07	1.97 ± 0.13

Values are given as mean ± SD; $n = 3$ replicate tanks.
[1] Specific growth rate – SGR.
[2] Apparent feed intake – FI, Body weight – BW.
[3] Feed conversion ratio – FCR.
[4] Protein efficiency ratio – PER.

employing Spirulina (*S. platensis*), Stanley & Jones (1976) reported a FCR of 2 in bigmouth buffalo, *Ictiobus cyprinellus* (a cypriniformes carp). In the present study, the feed conversion and protein utilization in the algae fed fish ranged from 1.43–1.7 to 1.66–1.97, respectively.

Statistical analyses of the proximate compositions of the whole fish and fillet revealed no significant differences, except in lipid contents (Table 8). Carp fed the higher level of MAP3 alga had significantly lower ($P < 0.05$) lipid content in whole fish than the control. In the case of fillet, difference between groups H3 and L8 was found to be statistically significant ($P < 0.05$).

Most studies on the application of algae in feeds of carps have focused on freshwater algae; we believe this to be the first report to examine the potential of commercially produced marine microalgal protein as a replacer of fishmeal protein in carp feeds.

Whiteleg shrimp

Whiteleg shrimp accepted all the test feeds readily, demonstrating the palatability of the new ingredients. Shrimp that were fed algae-based feeds did not differ from the control fishmeal-fed group in terms of their growth and feed performance (Table 9). However, some differences were noted in their body proximate composition (Table 10). As inclusion of 37% of tapioca in the diets contributed to better growth and feed conversion ratio in Indian white prawn *Penaeus (Fenneropenaeus) indicus* (Ali 1988), we have employed this as a filler.

The shrimp growth and feed performance data did not reveal any statistically significant differences during the entire period. However, body protein was lower ($P < 0.05$) at higher inclusion level of MAP8 compared to those of the control shrimp and L3. Group L8 had the highest lipid

Table 8 Proximate composition of the whole fish and fillet from carp offered microalgae-based feeds for 12 weeks

	Control	L3	H3	L8	H8
Whole fish					
Moisture	701.5 ± 18.9	718.6 ± 10.1	716.2 ± 15.4	701.9 ± 16.1	714.3 ± 15.0
Lipid	296.5 ± 7.3[a]	240.5 ± 2.6	211.8 ± 5.1[b]	288.3 ± 12.0	267.9 ± 5.5
Protein	580.4 ± 7.1	605.9 ± 6.0	592.9 ± 2.4	582.0 ± 3.0	583.6 ± 7.1
Ash	111.0 ± 10.4	114.7 ± 2.4	125.3 ± 8.5	108.5 ± 0.2	107.7 ± 7.5
Fillet					
Moisture	781.6 ± 4.7	779.2 ± 2.1	779.7 ± 7.4	779.1 ± 7.2	782.5 ± 4.5
Lipid	68.7 ± 2.2	71.9 ± 1.1	57.0 ± 0.4[a]	78.0 ± 0.6[b]	74.0 ± 1.8
Protein	855.2 ± 19.8	836.6 ± 2.6	861.5 ± 7.3	863.9 ± 7.3	857.8 ± 1.9
Ash	66.8 ± 3.8	62.3 ± 2.4	65.2 ± 2.4	61.8 ± 2.3	66.3 ± 2.7

Values (g kg^{-1} dry weight) are given as mean ± SD; $n = 3$ fish from each of the three replicate tanks.
Different superscripts indicate statistically significant differences ($P < 0.05$), if any, between the groups in a particular row.

Table 9 Survival, growth and feed utilization during a 9-week feeding trial on whiteleg shrimp offered microalgae-based feeds

	CO	L3	H3	L8	H8
Survival (%)	86.00 ± 9.25	90.67 ± 4.94	91.33 ± 1.83	91.33 ± 6.50	86.00 ± 4.94
Weight (g)					
Week 0	2.22 ± 0.01	2.21 ± 0.01	2.22 ± 0.01	2.22 ± 0.01	2.21 ± 0.01
Week 4	6.13 ± 0.38	6.31 ± 0.35	6.32 ± 0.14	6.45 ± 0.24	6.11 ± 0.31
Week 9	10.41 ± 1.16	11.02 ± 0.86	10.77 ± 0.52	10.50 ± 0.62	10.78 ± 0.82
Weight gain (%)	368.97 ± 51.51	398.31 ± 36.68	384.71 ± 22.38	374.12 ± 26.96	386.94 ± 36.68
SGR (% day^{-1})[1]	2.44 ± 0.18	2.55 ± 0.12	2.50 ± 0.07	2.47 ± 0.09	2.51 ± 0.12
FI (% BW day^{-1})[2]	4.90 ± 0.43	4.72 ± 0.17	4.89 ± 0.18	4.82 ± 0.20	4.81 ± 0.18
FCR[3]	1.91 ± 0.20	1.72 ± 0.12	1.81 ± 0.12	1.82 ± 0.05	1.81 ± 0.12
PER[4]	1.62 ± 0.16	1.69 ± 0.10	1.57 ± 0.11	1.60 ± 0.03	1.63 ± 0.10

Values are given as mean ± SD; $n = 5$ replicate tanks.
[1] Specific growth rate – SGR.
[2] Apparent feed intake – FI, Body weight – BW.
[3] Feed conversion ratio – FCR.
[4] Protein efficiency ratio – PER.

Table 10 Proximate composition of the whole shrimp offered microalgae-based feeds for 9 weeks

	CO	L3	H3	L8	H8
Whole shrimp					
Moisture	771.2 ± 7.1	777.8 ± 5.7	775.9 ± 6.4	777.6 ± 5.3	768.8 ± 6.7
Lipid	75.7 ± 1.4	73.2 ± 0.6[a]	72.1 ± 6.6	77.2 ± 0.3[b]	74.3 ± 5.0
Protein	740.5 ± 4.3[a]	743.8 ± 10.2[a,c]	737.0 ± 3.6	738.5 ± 0.6	702.9 ± 1.5[b]
Ash	109.9 ± 3.0	107.1 ± 9.8[a]	120.0 ± 3.8[b]	106.5 ± 2.4[a,c]	116.4 ± 2.7

Values (g kg^{-1} dry weight) are given as mean ± SD; n = 3 shrimps from each of the five replicate tanks.
Different superscripts indicate statistically significant differences ($P < 0.05$), if any, between the groups in a particular row.

content which was significantly ($P < 0.05$) greater than that of group L3. The ash content of shrimps increased with higher inclusion levels of both algae; the difference was significant for the MAP3 diet.

There is hardly any information on the use of microalgae as a dry feed component for shrimps, though there are ongoing efforts to replace fishmeal protein using terrestrial plant proteins. *L. vannamei* has been successfully grown on a predominantly plant-protein diet containing solvent-extracted soybean meal, corn gluten meal and corn fermented soluble, which together accounted for nearly 98% of the total dietary protein of 36% (Amaya *et al.* 2007a). The same research group has verified the concept of fishmeal-free shrimp feed in a pond trial (Amaya *et al.* 2007b). Furthermore, beneficial impact of algal inclusion on shrimp health has been reported recently – *L. vannamei* fed diets supplemented with marine algal meals rich in docosahexaenoic acid and arachidonic acid demonstrated significant improvement in immune responses (Nonwachai *et al.* 2010). The evidence from these studies indicate that algal meal that is capable of replacing fishmeal protein also has the potential to improve the health of shrimp. However, the latter aspect needs to be ascertained through additional studies.

Conclusion

These short-term studies have helped us to understand the potential of the microalgal meals as replacements for fishmeal in the feeds of Atlantic salmon, common carp and whiteleg shrimp. The two microalgae could be incorporated at levels tested in this study; some significant effects were noted on body composition as an effect of higher inclusions. Further studies are necessary to confirm the suitability of these ingredients in practical feed formulations.

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