FEED SUSTAINABILITY (/ADVOCATE/CATEGORY/FEED-SUSTAINABILITY)

Green water meal has potential as aquafeed ingredient

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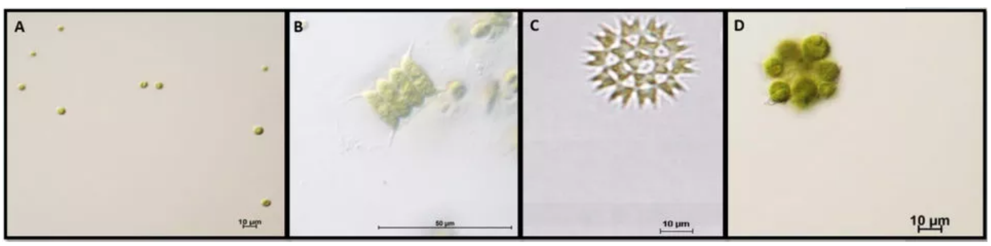
Current research looking at nutritional value and use in shrimp diets



Microalgae species found in the green water system, Borneo Marine Research Institute. (A) Chlorella sp.; (B) Scenedesmus sp.; (C) Pediastrum sp.; (D) Coelastrum sp. The proportion of microalgae species in the green water is not constant and slightly vary between days of production. However, Chlorella sp. was identified as the dominant microalgae species found in the green water production.

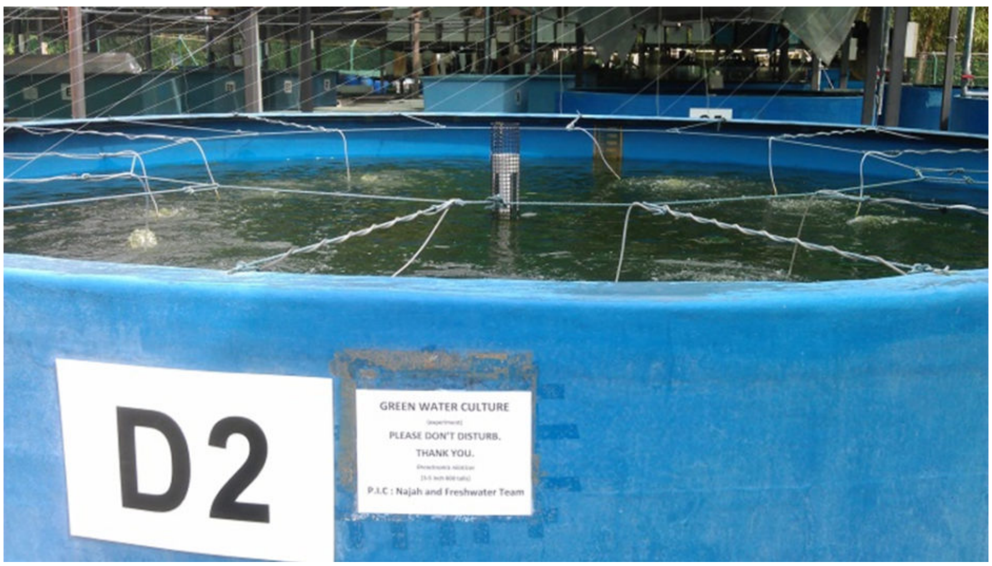
Microalgae – also known as phytoplankton – represent the largest group of photosynthetic microorganisms on earth. These simple, unicellular organisms can potentially yield large amounts of biomass within a short time, year-round.

In recent decades, microalgae have received much attention because of their fast growth rates, photosynthetic efficiency and higher biomass produced when compared to terrestrial crops. In the aquaculture industry, microalgae production is done under intensive or extensive monoculture, and various microalgae are commonly used as starter or grow-out feeds for shrimp, fish and bivalves. They can also be used to enrich other larval feeds like **rotifers** (https://www.aquaculturealliance.org/advocate/rotifers-diatoms-aid-shrimp-biofloc-nurseries/) or **artemia** (https://www.aquaculturealliance.org/advocate/artemia-the-magic-powder-fueling-a-multi-billion-dollar-industry/).

In addition to providing food for aquacultured organisms, microalgae also help improve the water quality. Introduction of microalgae into rearing tanks of a variety of freshwater and seawater animals – known as "green water systems" – typically result in improved survival rates and growth performance when compared to more traditional "clear water" systems. This is likely due to the water quality enhancement through higher oxygen production and more stable pH in the culture environment. Microalgae are also used as formulated aquafeed ingredients, as a source of protein or lipids, to improve animal growth and survival, to enhance pigmentation and also to reduce culture time.

Production of green water meal and its nutritional value

Production of green water in tilapia culture tanks is carried out continuously at our hatchery in the Borneo Marine Research Institute (Universiti Malaysia Sabah, Malaysia) as part of the facility's culture of freshwater fish such as tilapia and catfish. Here we summarize results of a study (*Journal of Applied Phycology*, doi:10.1007/s10811-014-0383-6) of production of green water, the nutritional value of the microalgae and the meal produced, and an experiment to replace fishmeal in diets for Pacific white shrimp (*Litopenaeus vannamei*).



The green water production tank.

A starter stock was prepared using about 1,000 liters of green water from another tilapia culture tank culture and 14,000 liters of freshwater, transferred to a production tank (20,000-L capacity). This 15,000-liter green water starter stock contained approximately 250,000 cells/mL of phytoplankton. Seven-hundred red tilapia (*Oreochromis* sp.) juveniles were stocked in the production tank and fed a commercial tilapia pellet (32 percent crude protein and 4 percent crude lipid, from Cargill, Malaysia) twice daily at 1 percent of their body mass to support a long and continuous culture period and produce a high density of microalgae in the tank.

Bloomed microalgae from the tank were harvested by pump and filtration, harvesting only particles in the sizer range of 0.1 to 60 m, which were used as the green water meal. The green water was harvested at its mid to late-logarithmic growth phase, based on the microalgae cell density.

At each harvest, about 10 percent (1,500 liters) of the green water volume in the tank was pumped out through the filter, which produced approximately 20 to 30 liters of concentrated green water. The harvest frequency was dependent on the microalgae cell density in the culture tank. For example, the green water was harvested only once a week in the heavy raining season, but in most cases, it could be harvested twice per week. The concentrated green water was stored in a cold room (minus-5 degrees-C) for about 3 to 4 days to allow it to sediment, and then the supernatant (about 70 percent) was eliminated and the microalgae biomass was collected and spray-dried.

The green water meal produced in our facilities contained 99.76 ± 0.05 percent dry matter, 39.03 ± 0.19 percent crude protein, 1.56 ± 0.11 percent crude lipid, 8.34 ± 0.50 percent crude fiber and 5.97 ± 0.17 percent ash. In general, the green water meal produced in tilapia culture tanks is characterized by high protein, low lipid and moderate fiber and ash contents.

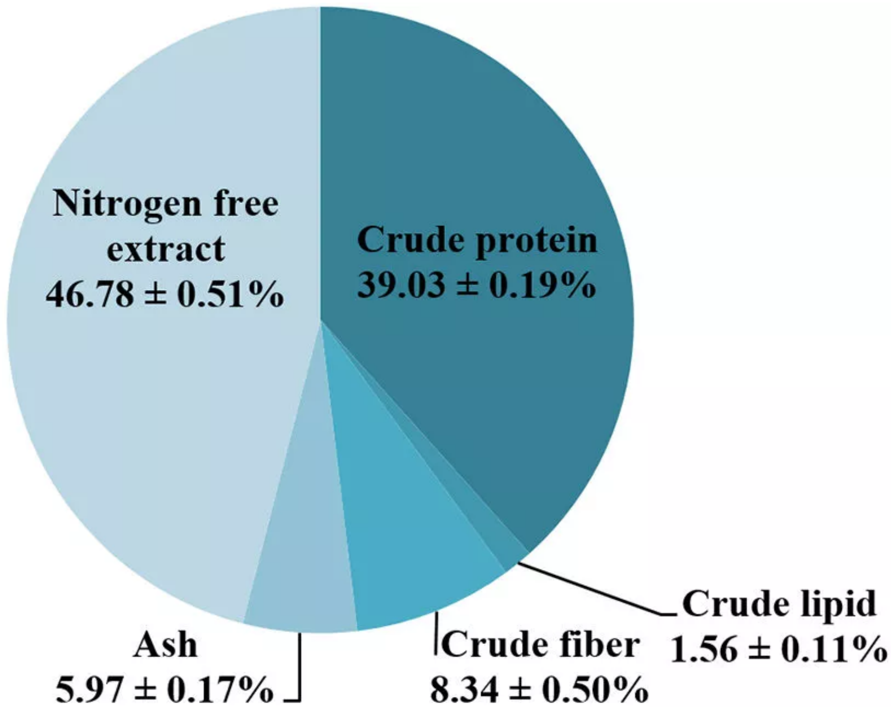


Fig. 1: Proximate composition of green water meal produced at the Borneo Marine Research Institute.

The nutritional value of microalgae may vary significantly in particular species, depending on the culture conditions. The phytonutrients of the microalgae produced included particular types of polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), arachidonic acid (AA) and docosahexaenoic acid (DHA). Typically, microalgae harvested in the late-logarithmic growth phase contain 30 to 40 percent protein, 10 to 20 percent lipids and 5 to 15 percent carbohydrates, but these values may differ significantly when cultivated at different phase. In general, most microalgal species contain moderate to high percentage of EPA (7 to 34 percent). *Pavlova* spp., *Isochrysis* sp. and other cryptomonads are mostly rich in DHA (0.2 to 11 percent), whereas *Nannochloropsis* spp. and diatoms have the highest percentages of AA (up to 4 percent).

Several species of microalgae can be found in green water systems, mostly from the Chlorophyta division. Because Chlorophyte microalgae typically lack PUFAs, they are considered unsuitable as single species for use in aquaculture diets. Instead, a mixture of microalgae species should be given to the cultured organisms to provide better nutritional value and thus effectively improve the growth performance. The gross chemical composition of some common microalgae species used in aquaculture industry is listed in Table 1.

Shapawi, Green water meal, Table 1

Microalgae species	% dry matter: M	% dry matter: CP	% dry matter: CL	% dry matter: CF	% dry matter: Ash	% dry matter: NFE	R
Chaetoceros calcitrans	–	36.4	15.5	–	14	–	A
Chlorella sp.	–	30.7	17.9	–	7.9	–	B
Chlorella spp.	5.99	46.78	14.83	8.32	18.75	11.32	C

Chlorella vulgaris	–	55.0	10.2	5.8	5.8	23.2	D
Chlorella vulgaris	3.6	52.8	8.1	20.8	9.13	5.57	E
Chlorella vulgaris	–	38	5	–	–	–	F
Cladophora glomerata	1.6	31.6	5.2	11.2	23.6	28.4	G
Dunaliella salina	–	40.3	28.1	–	9.0	–	B
Dunaliella salina	–	40.21	18.02	2.1	15.89	–	H
Hydrodictyon reticulatum	5.7	27.7	1.9	14.9	32.6	22.9	I

Table 1. Proximate composition of some common microalgae species.

M= moisture, CP= crude protein, CL= crude lipid, CF= crude fiber, NFE= nitrogen free extract (100-CP-CL-CF-Ash), R= references (available from first author).

The microalgae species identified in our green water system include *Chlorella* sp., *Scenedesmus* sp., *Pediastrum* sp. and *Coelastrum* sp. The proportion of microalgae species in the green water is not constant and can vary slightly between days of production. However, *Chlorella sp*. was the dominant microalgae species found in our green water production system.

Fishmeal replacement for Pacific white shrimp

We carried out an experiment with various levels of inclusion of green water meal as fishmeal replacement in experimental diets for Pacific white shrimp. Table 2 shows the growth performance of shrimp fed the experimental diets. The weight gain was significantly influenced by the inclusion level of green water meal in the diets.

In general, shrimp growth decreased with increasing level of green water meal substitution. The control diet (GWM0) resulted in the highest weight gain (292.56 ± 25.63 percent), followed by GWM10 (247.22 ± 11.97 percent), GWM20 (212.82 ± 12.69 percent), GWM30 (186.16 ± 10.04 percent), and GWM40 (112.51 ± 11.09 percent). Unlike weight gain, the specific weight gain (SGR) of GWM10 was not significantly different from the control diet. However, other diets yielded significantly lower SGR than the control diet. Except for GWM40, the dry feed intake of GWM-based diets was not significantly different from the control diet.

Shapawi, Green water meal, Table 2

Diets	Initial weight (g)	Final weight (g)	Weight gain (%)	SGR (%/day)
GWM0	1.74 ± 0.01a	6.81 ± 0.25d	292.56 ± 14.80d	3.03 ± 0.06d
GWM10	1.73 ± 0.03a	5.99 ± 0.21c	247.22 ± 6.91c	2.75 ± 0.08cd
GWM20	1.72 ± 0.02a	5.37 ± 0.12bc	212.82 ± 7.33bc	2.47 ± 0.02bc
GWM30	1.73 ± 0.01a	4.93 ± 0.07b	186.16 ± 5.8b	2.31 ± 0.03b

GWM40	1.73 ± 0.01a	3.69 ± 0.10a	112.51 ± 6.4a	1.67 ± 0.08a

Growth performance of shrimp fed the experimental diets. Mean values with different superscript within a column are significantly different ($p<0.05$).

Table 3 shows the feed utilization of shrimp fed the experimental diets. Feed conversion ratio (FCR) followed the SGR trend where FCR of GWM10 (1.87) was not significantly different from the control diet (1.59). Meanwhile, FCR values in other diets were above 2.1, and these values were significantly poorer than the FCR of the control diet. Protein efficiency ratio (PER) and apparent net protein utilization (ANPU) were ranged from 0.63 to 1.51 and 10.60 to 24.51, respectively. The values of PER and ANPU decreased as the inclusion of green water meal in the diets increased. The survival rate of the shrimp fed with experimental diet was above 95 percent.

Shapawi, Green water meal, Table 3

Diets	DFI (g/shrimp)	FCR	PER	ANPU (%)
GWM0	8.05 ± 0.09b	1.59 ± 0.06a	1.51 ± 0.06d	24.51 ± 1.15d
GWM10	7.92 ± 0.12ab	1.87 ± 0.10ab	1.30 ± 0.07cd	23.29 ± 1.15cd
GWM20	7.98 ± 0.19ab	2.19 ± 0.06b	1.10 ± 0.03bc	19.66 ± 0.62c
GWM30	7.68 ± 0.04ab	2.40 ± 0.05b	1.00 ± 0.02b	15.55 ± 0.34b
GWM40	7.44 ± 0.13a	3.82 ± 0.22c	0.63 ± 0.04a	10.60 ± 0.66a

Feed utilization of shrimp fed the experimental diets.

Mean values with different superscript within a column are significantly different ($p<0.05$).

A distinct difference in intense red/orange coloration of the shrimps fed with the experimental diets is shown in Fig. 2.

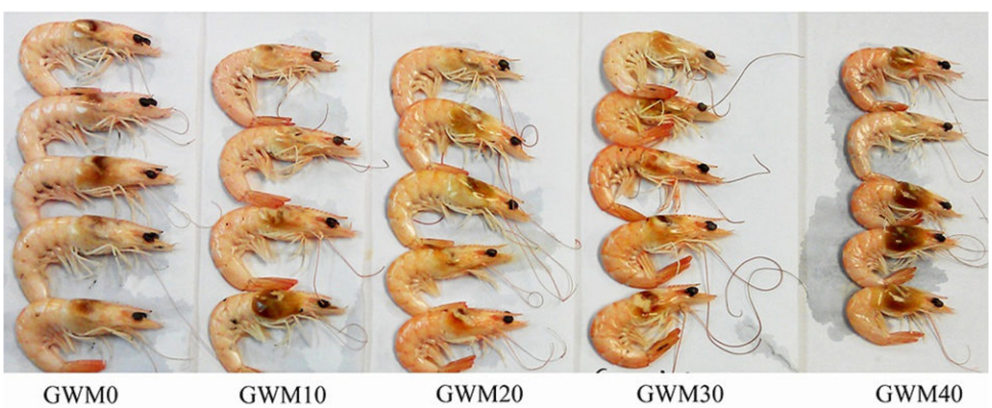


Fig. 2: Color of shrimp fed the experimental diets, after boiling following harvest.

Shrimp fed with GWM-based diets appeared to be redder/more orange when compared to the control diet. This is supported by the significantly higher ($p<0.05$) carotenoid concentrations in GWM-based diets than in the control diet (GWM0), as shown in Fig. 3.

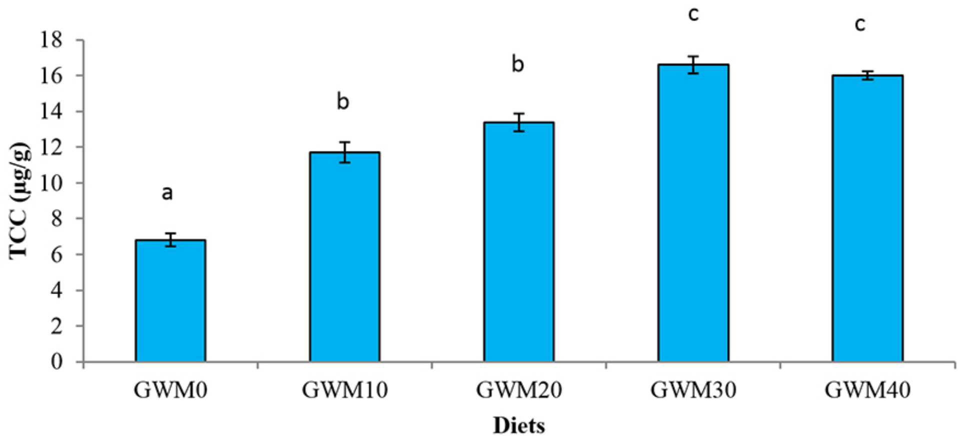


Fig. 3: Total carotenoid concentration, TTC (g/g) in the shrimp at the end of the experiment.

Shrimp fed with GWM0 diet only contents 6.81 ± 0.36 µg/g of total carotenoid concentration. While shrimp fed with the GWM30 diet contains the highest total carotenoid concentration (16.60 ± 0.48 µg/g). Feeding shrimp with GWM-based diets increased the carotenoid contents of the shrimp by about 72 percent (GWM10), or 11.71 – 16.6 µg/g in GWM-based diets compared to 6.81µg/g in the control diet.

Perspectives

Green water production systems provide food for aquacultured organisms and also improve overall water quality. Microalgae from these systems can be used to produce green water meal, an aquafeed ingredient that has shown potential in experiments to partially replace fishmeal in practical diets for Pacific white shrimp, and also to enhance desirable shrimp pigmentation.

References available from the first author.

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