SUITABILITY OF SEAWEED MEAL INCORPORATED WITH *RHODOVULUM* SP. BACTERIUM AS FEED SUPPLEMENT FOR FINFISH LARVAE

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ABSTRACT. *The proximate compositions of three Chlorophyta (Caulerpa sp., Halimeda macroloba, and Halimeda opuntia), three Phaeophyta (Sargassum sp., Padina minor, and Dictyota sp.), and one Rhodophyta (Laurencia sp.) were determined in order to evaluate their suitability for feed supplement. The evaluated seaweed showed variable protein content between 4 to 14% (dry weight). The highest protein level of 14% dry weight (lDW) was determined in Sargassum sp., but this species also recorded the lowest level (0.2-0.5%) of lipid. On the other hand, the highest content of 4% (DW) lipid and 11% (DW) protein level was observed in the species Dictyota. Of the other species, Padina minor from the same group, shows 10% (DW) and 1% (DW) levels of protein and lipid respectively. Seaweed meal of these three species was prepared and mixed separately with 2% of Rhodovulum sp. Thus, three types of supplementary diet were prepared for Tilapia fry feeding trials. Rhodovulum sp. bacterium biomass alone was used as control diet. The preliminary feeding trial showed that the growth and survival of fin fish larvae were improved with the supplemented diet made from Dictyota meal mixed with 2% of Rhodovulum sp. Thus, indigenous self-flocculated phototrophic bacterium Rhodovulum sp. is improved when used as a feed additive enhanced with this seaweed meal in Tilapia (Oreochromis niloticus) fry rearing period.*

KEYWORDS. Growth, *Rhodovulum* sp., seaweed meal, survival, Tilapia fry.

INTRODUCTION

Global seaweed production is increasing and is mainly used for food additive and vegetable production. The protein content of seaweed differs according to species and environment. Seaweed is rarely promoted for the nutritional value of its protein. At present, seaweed with elevated protein content and production rate is receiving increasing attention as a novel food with potential benefits (Buschmann *et al*. 2001) and as a possible ingredient in fish diets (Davies *et al*. 1997). The cultivation of seaweed on a commercial scale remains restricted to a few species (Buschmann *et al*. 2001). There is very little study on the proximate composition of seaweed found in Malaysia (Matanjun *et al.*, 2009). This is mostly because seaweed as a food source in Malaysia is still not as common as in Japan and USA (Hawaii).

Seaweed meal is used as a feed ingredient o reduce cost, and to increase the growth and survival of fish species. In aquaculture, seaweed with a high protein level is used in the production of food for fish. Seaweed (*Gracilaria bursa-pastoris, Ulva rigida,* and *Gracilaria cornea*) was proved to have great potential as an alternative ingredient in diet for European sea bass juveniles as there was improvement in growth performance (Valente *et al*., 2006). Purple non-sulfur bacteria (PNSB) could also be used to supplement feed along with seaweed meal. The fresh PNSB biomass is not only rich in high quality protein, but also contains significantly large amounts of carotenoid pigments, biological co-factor and vitamins. In addition, the biomass is found to be rich in enzymes and active compounds (Kobayashi & Kobayashi 2001). The bacterium *Rhodovulum sulfidophilum,* when combined with commercial tilapia feed, improves the growth and survival of tilapia during grow out period (Banerjee *et al*. 2000). *Rhodovulum* sp. contains protein, lipids, vitamins, minerals, carotenoids and other co-factors, which have potential application as feed enhancer (Azad *et al*., 2001, 2002). The biomass, when added to other feed, improves water quality (Azad *et al*., 2002), increases grazing ability and reduces the metamorphosis period in shrimp larvae (Azad *et al*., 2002).

Although several studies were conducted to evaluate the replacement of fish meal with plant ingredients in fish feed, data about the potential use of seaweed in fish diet is scarce (Davies et al. 1997). Further, there is a lack of information about the seaweed meal as supplementary feed incorporated with bacterial biomass. This experiment was undertaken with an aim to evaluate the proximate composition of locally collected seaweed and its suitability, when incorporated with *Rhodovulum* sp, to enhance the growth and survival of swim up tilapia (*Oreochromis niloticus*) fry.

METHODOLOGY

Seaweed meal

Seven types of wild seaweed were collected from Banggi Island (7.14ºN, 117.10ºE) which is situated within the Kudat Division, off the northern tip of Sabah, East Malaysia. In the laboratory, seaweed was identified to genus and species level based on examination of morphological characteristics (FAO, 1998). Collected varieties of seaweed were washed with distilled water and placed under normal sunshine for complete dryness. Based on their proximate composition three of them were selected and ground into powder to form seaweed meal.

Rhodovulum sp. biomass

Fresh bacterial biomass of the purple non-sulfur phototrophic bacterium strain *Rhodovulum* sp. was grown in synthetic 112 media. The bacterium *Rhodovulum* sp. was isolated locally from UMS sea bass brood stock tanks. The bacterium was grown in a five liter capacity cultured bottle under 2500 lux illumination intensity with 30±2^{o}C temperature in anaerobic conditions. Indigenous *Rhodovulum* sp. self-flocculated and on the third day the culture was taken for experimental diet. The fine powder seaweed meal was thoroughly mixed with 2% (w/w) fresh bacterial biomass of *Rhodovulum* sp.

Experimental fish and preliminary trial protocol

The preliminary feeding trial was carried out at the wet laboratory of Borneo Marine Research Institute, University Malaysia Sabah (UMS), Kota Kinabalu. The swim up tilapia fry (*Oreochromis niloticus*) were obtained from the UMS hatchery. Ten litre capacity plastic aquariums with working volume of eight litres were used as culture units in a recirculation system. Each aquarium was filled with seven litres of filtered freshwater and aeration was provided with an air stone from a central air blower system. Equal sizes of active and healthy tilapia swim up fry were selected for stocking. Groups of 30 fish (mean weight 0.02±0.01) were randomly distributed into 12 aquariums (three replicates for each of the diets).

Feed and Feeding

Four supplemented diets were made from three selected seaweed meal. Diets were *Rhodovulum* sp. (Rv) biomass (Diet 0), Rv.+ *Sargassum* sp. (Diet 1), Rv.+*Padina minor* (Diet 2) and Rv.+ *Dictyota* sp (Diet 3). Fish were fed twice a day (8.00 a.m. and 4.00 p.m.). Diets were distributed by hand and fish were allowed to take feed until satiation. Daily feed allowance was adjusted accordingly. In addition to experimental diets, everyday one litre of green water was added after removal of one litre of water from the aquarium. The excess feed was siphoned out one hour after feeding using a bottom cleaner. The growth (g) and survival (%) of tilapia (*Oreochromis niloticus*) were monitored for the period of 60 days.

Analytical parameters

Seaweed

Proximate compositions (%) of seaweed including moisture and ash were determined (AOAC, 1990). Crude protein, crude lipid and crude fibre were determined by Kjeldahl (Kjeldahl System 1026 Manual, 1987), Soxlet (Tecator Soxlet Manual, 1983) and Fibre Tech Analyzer (Tecator fibre tech manual, 1983) respectively. The methods were modified by Foss Analytical Manual AB (2003).

Feeding trial

Insitu parameters, such as water temperature (˚C), dissolved oxygen (mg/L) and pH were measured every day with multi-probe analyser Hanna HI 9828. Every fifteen days all fish were caught with a scoop net from the each aquarium and put in a one litre aquarium filled with freshwater and 2 drops of anaesthetic, *Nika transmore*. The fish were left for few minutes until they moved slowly and individual weight (g) of 20 fish was taken using an analytical balance to monitor the increment of growth. Finally fish were counted to determine the percentage of survival in each experiment unit.

Statistical analysis

The quantitative data was presented as mean ± standard deviation (SD) of three replicated diets. One way variance analysis was performed with Statistica 6.0 (windows package) to compare the growth and survival in tilapia with different types of supplemented diets.

RESULTS

Seaweed

A total of 7 species of seaweed (3 from phylum Chlorophyta, 3 from phylum Phaeophyta, and 1 from phylum Rhodophyta) were collected and identified. Identified species are *Sargassum* sp., *Caulerpa* sp., *Padina minor*, *Halimeda macroloba*, *Halimeda opuntia*, *Laurencia* sp., and *Dictyota* sp.

Table 1. The proximate composition of seaweed meal collected locally and *Rhodovulum* sp. used in the preliminary feeding trial

	Moisture (%)	Ash (%)	Lipid (%)	Protein (%)	Fiber (%)	NFE (%)
***Sargassum* sp.**	**81.7**	**24.88**	**0.48**	**13.85**	**7.58**	**53.21**
***Caulerpa* sp.**	**87.29**	**32.63**	**0.48**	**10.61**	**7.4**	**48.88**
Padina minor	**86.56**	**33.33**	**1.47**	**10.04**	**5.42**	**49.72**
Halimeda macroloba	**75.34**	**61.5**	**0.2**	**6.79**	**3.79**	**27.72**
Halimeda opuntia	**54.48**	**86.67**	**0.24**	**3.84**	**1.38**	**7.86**
***Laurencia* sp.**	**91.7**	**23.78**	**0.29**	**5.99**	**5.49**	**64.45**
***Dictyota* sp.**	**85.18**	**26.19**	**3.90**	**10.37**	**7.61**	**51.93**
***Rhodovulum* sp.**	**6.71**	**14.32**	**5.9**	**62.3**	**0.5**	**10.57**

Most of the seaweed had high moisture content, which comprised approximately an average 70% of wet weight. The moisture content (by wet. wt) of *Sargassum* sp., *Caulerpa* sp., *Padina minor*, *Halimeda* and *Dictyota* sp. ranged from 55 to 92 %. *Sargassum* sp. had higher crude protein content than *P. minor*, and *Dictyota* sp., but all those were in the range of 10 – 14 % of dry weight. On the other hand, the lowest crude protein was obtained from *H. macroloba*, *H. opuntia*, and *Laurencia* sp. in the range of 5 – 7%, 3 – 4 %, and 5 – 6 % of dry weight respectively. The lipid content of most species of seaweed was low (all < 1% d. wt) except for *Dictyota sp.* (4% of dry weight). The crude lipid content of *Caulerpa* sp., *Padina minor* and *Sargassum* sp. was 0.5 %, 1.5 %, and 0.5 % respectively. Ash was the most abundant component of dried material in *H. macroloba* and *H. opuntia*, which ranged from 61 – 87 %. While for *Sargassum* sp., *Caulerpa* sp., *P. minor*, *Laurencia* sp., and *Dictyota* sp., the ash content was in the range of 24-33 % of dry weight.

Water quality parameters

The water quality parameters in all the culture units were almost same and within the range of 29-30^{o}C, 8.5-8.5mg/L and 7.8-8.4 for temperature, dissolved oxygen and pH respectively.

Growth and survival

The highest growth of 1.96±0.06g with Diet 3 (Rv. + *Dictyota*) was significantly different (p<0.05) from the other experimental diets. The growth was enhanced with Diet 3 from the start of the experiment to the end of the feeding trial (Fig. 1).

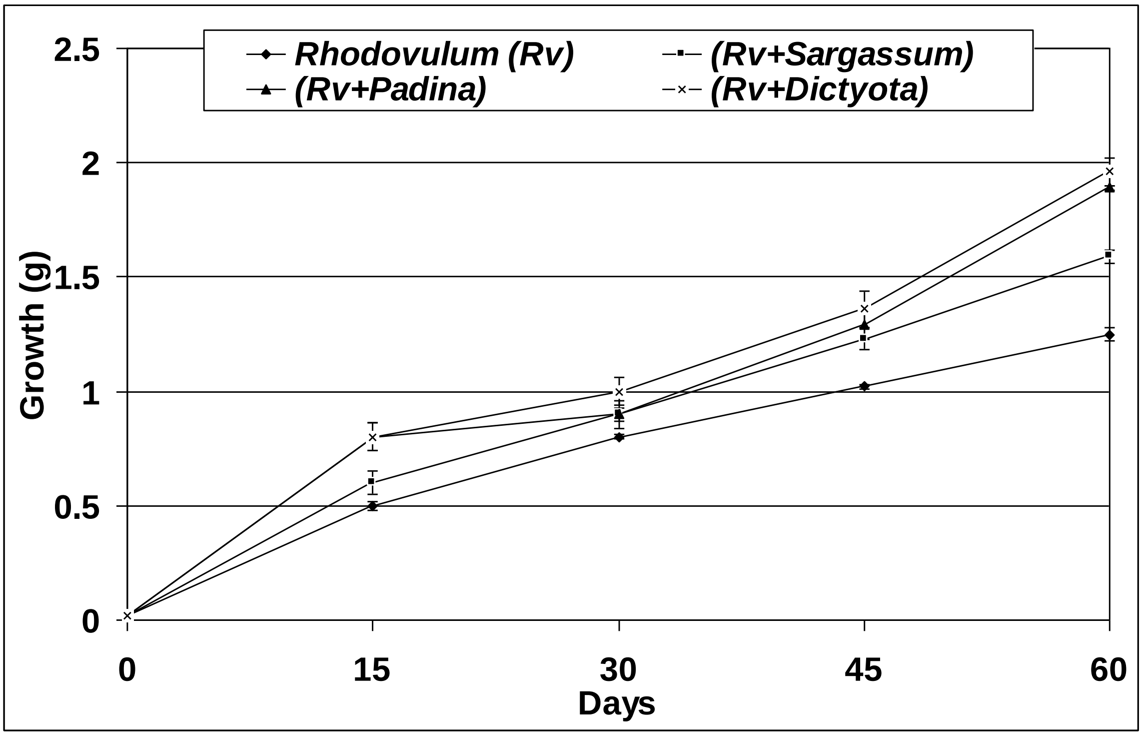

Figure 1. Growth (g) in *O. niloticus* with the four types of experimental diet during 60 days feeding trial

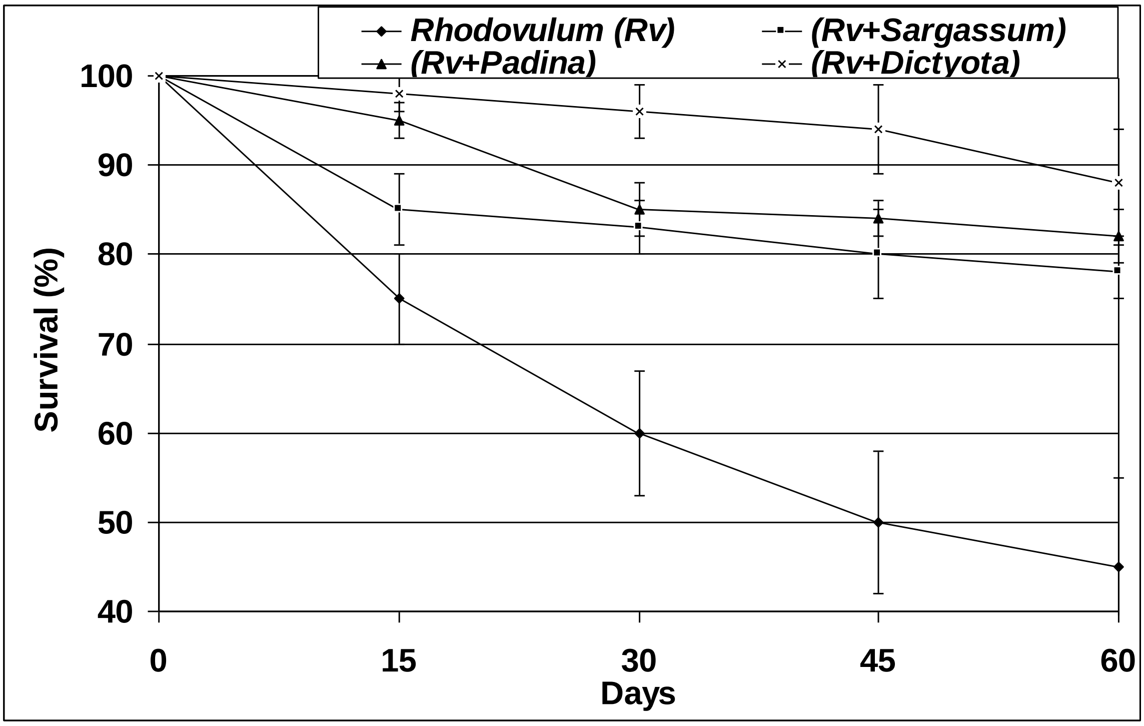

Figure 2. Survival (%) of *O. niloticus* fed with four types of experimental diet during 60 days feeding trial

The highest survival of 88% and the lowest of 45% were observed in tilapia (*Oreochromis niloticus*) fry fed with Diet 3 (Rv. + *Dictyota*) and Diet 0 (*Rhodovulum* sp. biomass) respectively (Fig.2) similar to the growth rate pattern. Significant differences (P<0.05) were observed in survival of *O. niloticus* fed with four types of experimental diet.

DISCUSSION

Nutritional properties of seaweed are not as well known as those of land plants, however, they are low in lipids but rich in proteins, non-starch polysaccharides, minerals and vitamins. Seaweed contains a large percentage of moisture, which may range from 68.0 to 94.3 % (McDermid & Stuercke 2003) of wet weight. The moisture content (by wet. wt) of wild *Sargassum* sp., *Caulerpa* sp., *Padina minor*, *Halimeda* and *Dictyota* sp. in this study ranged from 55 to 92 %. The moisture content of *Laurencia* sp., *Sargassum* sp., and *Dictyota* sp. collected from Hawaii was within the range of 60 to 95% (McDermid & Stuercke, 2003). The protein content of seaweed differs according to species. The protein content of each species varies depending on the seasonal and environmental growth conditions (Dawczynski *et al*., 2007). Generally, the protein fraction of brown seaweed was low (3 – 15 % dry weight) compared with that of the green or red seaweed (10 – 47 %) (Fleurence 1999). In this study, *Sargassum* sp., *Caulerpa* sp. *Padina minor* and *Dictyota* sp. had protein content higher than 10 % of dry weight, which was similar to that reported by McDermid & Stuercke (2003). Higher protein content was recorded for red seaweed such as *Porphyra tenera* (47 % dry weight) and *Palmaria palmata* (35 % dry weight) (Fleurence, 1999). Galland-Irmouli *et al*. (1999) showed that the protein content of *Palmaria palmata* varied from 9 – 25% with a yearly average of 18± 6 %. Generally, the lipid content of seaweed was less than 4 % (Wong & Cheung, 2000). The lipid content of the seaweed observed in this study was in the range of 0.2 to 4 %. The observed values were much lower than the expected values from other studies. The highest, with 4% lipid, was observed from *Dictyota* sp. in this study. Two species, *Dictyota sandvicensis* (20 % dry weight) and *D. acutiloba* (16%) recorded markedly high crude lipid content (McDermid & Stuercke, 2003).

In aquaculture, high protein seaweed is used in the production of feed for fish. Seaweed (*Gracilaria bursa-pastoris*, *Ulva rigida*, and *Gracilaria cornea*) is proved to have great potential as an alternative ingredient in diet for European sea bass juveniles as it improves growth performance (Valente *et al.*, 2006). The seaweed meal they used as diet ingredient was different from this study. In this study the highest growth and survival of tilapia fry was obtained with *Dictyota* species incorporated with biomass of *Rhodovulum* sp. The proximate composition of *Dictyota* species was much better than the other two species tested, as it contained a higher percentage of lipid. The addition of lipid in diets essentially improves the growth and survival of fish larvae. The nutritive value of different species of seaweed may play a role in the preference. In addition, the growth might be enhanced by the use of *Rhodovulum* sp. The utilisation of seaweed diets depends on the degree of digestion and limitation on the percentage of seaweed meal required to formulate the diets. Growth and protein utilisation efficiencies in *Oreochromis niloticus* and *Tilapia zillii* increased when fed diets containing 5% of fresh algae *Hydrodictyon reticulatum*, but growth reduced with increasing dietary inclusion levels (Appler, 1985). The increasing incorporation of both seaweeds *Gracilaria* and *Ulva,* from 5% to 10% did not affect growth performance or feed efficiency of European Seabass (Valente *et al*., 2006). Snakehead (*Channa striatus*) fry showed that the incorporation of 5% *Ulva* resulted in increased growth rate, feed efficiency and feed consumption, but the same level of *Gracilaria* spp. had no significant effect on those parameters (Hashim & Mat Saat, 1992). An experiment in seed production of *Penaeus chinensis* fed with a mixture of four strains of *Rhodopseudomonas* sp. improved grazing ability of shrimp (Cui *et al*., 1997). Although this study did not cover digestion and grazing ability, the growth and survival was improved when tilapia were fed with the experimental diets. Further the biomass of these types of bacteria could be used as aquaculture feed supplement (Banerjee *et al*., 2000) and also as feed additive in rearing shrimp larvae (Azad *et al*., 2002). The self-flocculated bacterium *Rhodovulum* sp. is not only rich in high quality protein, but also contains significantly large amounts of carotenoid pigments, biological co-factor and vitamins that might enhance the growth and survival in fish (Kobayashi & Kobayashi, 2001). However, all these parameters were not taken into account in this study. Addition of 1% *Rhodovulum sulfidophilum* bacterium biomass with *Skeletonema costatum* supported better growth and survival of *P. monodon* larvae reared from the naupliar to postlarval stage. Addition of more than 2% bacterial cells with the live feed did not support good growth as the water quality deteriorated (Azad *et al*., 2002).

Several varieties of seaweed, including *Ecklonia maxima*, *Laminaria japonica*, *Ulva rigida*, *Gracilaria gracilis*, *Ulva lactuta* were used for abalone culture. This seaweed was grown commercially for human consumption. The potential use of macro algae in fish feed depends on costs involved in their production, harvesting and processing prior to their inclusion in fish diets. Relatively low costs were incurred in seaweed production, as it is considered a by-product of the aquaculture industry, used as phytoremediation in the process to treat wastewater. Commercial utilisation of *Sargassum* sp., *Caulerpa* sp., *Padina minor* and *Dictyota* sp. is limited. These might be potential resources for aquaculture. More comprehensive research is however, needed to evaluate the efficacy of such products in longer term feeding trials and to determine the optimum dietary inclusion levels of seaweed in fish diets. Detailed nutritional profiling of the seaweed meal, especially the amino acid and fatty acid profiles are essential before utilising these non-conventional resources.

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