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Effect of *Spirulina platensis* and Probiotics as Feed Additives on Growth of Shrimp *Fenneropenaeus chinensis*

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Abstract The effect of *Spirulina platensis* and probiotics as feed additives on the growth of the shrimp *Fenneropenaeus chinensis* was investigated in comparison with a control. The shrimp were cultured in rearing tanks in a seawater pond for 35 days from September 1, 2004. As regards the water quality, the probiotic treatment (T2, commercial diet and 3% probiotics) produced a lower TDN (total dissolved nitrogen) and TDP (total dissolved phosphorus), making it effective in water quality improvement. Nonetheless, the phytoplankton flora succeeded from diatoms to cyanobacteria, regardless of the feed additives. Treatment T3, including 3% *S. platensis*, produced the highest mean body weight, which was 39% higher than that for all the other treatments ($P<0.05$). Accordingly, it was found that the use of *Spirulina* and probiotics as feed additives increased the shrimp body weight and improved the water quality, respectively.

Key words: Feed additive, *Fenneropenaeus chinensis*, phytoplankton, probiotics, *Spirulina platensis*

The shrimp *Fenneropenaeus chinensis* is an economically important aquaculture organism in the Yellow Sea coastal waters of Korea. The shrimp are reared to a commercial size through intensive culturing for a short period of time in a high water temperature. However, the dense stocking, waste concentration, and excess feed result in a poor water quality, allowing pathogenic organisms to become prevalent in the culture ponds. White spot syndrome virus (WSSV) is the most fatal source of infection for Asian cultivated shrimp [2], and since the first reports in Korea in 1993, the virus has continued to cause annual devastation of *F. chinensis* cultures. This high mortality of shrimp would seem to be related to the absence of an adaptive immunity in invertebrates like shrimp [15], as instead of an immunological memory, shrimp exhibit a phagocytic activity of hemocytes and humoral factors [10, 11, 29]. Therefore, enhancing the phagocytic activity in shrimp may help increase their survival rate and stabilize the farming.

The microalga *Spirulina platensis* (Cyanophyceae), which is rich in nutrients, with a high proportional protein content per cell, vitamins, essential amino acids, and fatty acids, as well as bioactive pigments, is already used as a human health supplement [3]. In addition, an enhanced immune activity or improved survival rate when using *S. platensis* as a food additive has also been reported for the aquaculture of certain fish: juvenile tilapia [27], Korean rockfish [6], catfish [8], and common carp [20]. In particular, to enhance the phagocytic activity of shrimp (*Penaeus merguensis*), Lee *et al.* [17] used the algae as a feed additive and reported that *S. platensis* (0.3% w/w feed) enhanced the phagocytic activity of shrimp hemocytes, and the activated phagocytic hemocytes then exhibited a greater capacity to engulf foreign agents, such as bacteria, plus a higher rate of phagocytosis. *S. platensis* has also been used to improve the water quality in shrimp culture ponds [7].

Probiotics, defined as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" [9], have recently been used to improve the survival rate and production yield of shrimp based on water quality improvement [23, 24]. Rengpipat *et al.* [25] reported that probiotic treatment significantly improved the survival rate and outer appearance of shrimp cocultured with the shrimp pathogen *Vibrio harveyi* for 10 days. Thus, for shrimp cultures, improving the quality of

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the rearing water and enhancing the immunity of the shrimp by probiotic treatment are likely to have a positive effect on their survival rate and production yield.

A successful shrimp culture clearly relies on maintaining an appropriate water quality, as a poor water quality can be directly linked to the outbreak of disease through infection from pathogenic organisms. Phytoplankton plays an important role in improving and maintaining the water quality in shrimp cultures [5, 12, 31]. Thompson *et al.* [28] previously reported that pennate diatoms and filamentous cyanobacteria are responsible for the largest uptake of ammonium from water in intensive shrimp cultures. Despite the important role of phytoplankton in water quality, its flora and fluctuations have not been extensively researched in the context of Korean shrimp culturing.

Accordingly, the present study investigated the effect of *S. platensis* and probiotics as feed additives on the growth of the shrimp *F. chinensis*. The phytoplankton flora was also surveyed, along with the basic nutrients (total dissolved nitrogen and total dissolved phosphorus) and physical data of the rearing seawater.

MATERIALS AND METHODS

Culture Tank and Shrimp

The shrimp culture was conducted in a static water pond adjacent to the coast at Umock-ri, Anjwa-myon, Sinan-gun, Jeollanam-do. Cylindrical fiberglass-reinforced plastic tanks (1-m diameter, 2-m height) containing 1 ton of seawater were used with seawater provided from a reservoir pond. The tanks were positioned in a row and connected to rectangular steel pipes supported by two rows of buoys. The settled tanks were then submerged, leaving the tank neck (0.4-m diameter, 0.3-m height) in the seawater reservoir pond.

The shrimp stocks were precultured with specific commercial diets in the static pond, and mid-sized shrimp grown to an 8.0-g body weight was used for the tank culture with a stocking density of 10 shrimp per tank. The culture tanks were continuously aerated without changing the seawater for 35 days from September 1, 2004.

Experimental Treatments

The *S. platensis* NIES 46 was provided by the National Institute for Environmental Studies (Japan) and grown in a SOT medium with the following composition: 16.8 g $NaHCO_3$, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$, 1 g K_2SO_4, 1 g NaCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.04 g $CaCl_2{\cdot}2H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.08 g Na_2EDTA, 0.03 mg H_3BO_3, 0.025 mg $MnSO_4{\cdot}7H_2O$, 0.002 mg $ZnSO_4{\cdot}7H_2O$, 0.0079 mg $CuSO_4{\cdot}5H_2O$, and 0.0021 mg $Na_2MoO_4{\cdot}2H_2O$, in 1 l of distilled water. The *S. platensis* was mass-cultured in a 200-l photobioreactor containing 100 l of the medium under continuous illumination at 100 μmol photons m^{-2} s^{-1} and air-lifted aeration for half a month, and then harvested with a sieve (20 μm pore-sized) and freeze-dried. The *Spirulina* powder was then mixed with a commercial diet (Vision Shrimp, Jeil Feed Co., Ltd., Korea) using 5–8% spraying water, followed by drying in a dry oven at 60°C for 1 h. The commercial diet (EP, extruded pellet) was composed of over 38% crude protein, over 7.0% crude lipids, below 4.0% crude fiber, below 17.0% ash, and 1.2% calcium, with 3.0% vitamins and minerals. The probiotics (Marinebio, Probionic Co., Ltd., Korea), a concentrated bacteria mixture, were provided without preculture according to the manufacturer's instructions. The commercial diet without any additives was used as the control (T1), and the following feed additives were tested: 3.0% probiotics (T2), 3.0% *S. platensis* (T3), 1.5% probiotics plus 1.5% *S. platensis* (T4), and half the basal diet with 1.5% *S. platensis* (T5), based on a dry weight relative to the commercial diet (Table 1). One g of feed was provided three times a day.

Table 1. Composition of diet and ratio of additives to 1 g of commercial feed.

Treatment	Commercial feed	Feed additive (w/w)
T1 (control)	100%	-
T2	97%	3% probiotics
T3	97%	3% *S. platensis*
T4	97%	1.5% probiotics +1.5% *S. platensis*
T5	Half volume of T3	

Analytical Methods

The temperature, salinity, and pH were measured *in situ* 7, 14, 21, and 35 days after beginning the experiment. To monitor the nutrients, such as the TDN (total dissolved nitrogen) and TDP (total dissolved phosphorus), and phytoplankton flora, seawater samples were collected 30 cm below the surface on the same days as above and transported in a state of cool preservation to the laboratory for further analysis. The analysis of each nutrient was conducted following APHA methods [1], and the biomass fluctuation of the phytoplankton was measured based on the chlorophyll *a* concentration [26]. To analyze the standing crop, species composition, and dominant species in the phytoplankton community, 500 ml of each seawater sample was fixed with Lugol's solution and concentrated to 40 ml. One ml of the concentrated sample was then used for species identification and cell counting under a microscope. To determine the effect of each feed, the final mean body weight and survival rate of the cultured shrimp in each tank were measured and compared at the end of the culture. A statistical analysis using a one-way ANOVA and multiple comparison (SPSS, Ver. 10.0 for Windows) with significance at $P<0.05$ was used to determine the differences in the shrimp mean body weight at the end of the experiment.

Table 2. Change in water temperature, salinity, and pH in a shrimp-rearing tank during experiment.

Treatment	Day 7			Day 14			Day 21			Day 35		
	Temp. (°C)	Salinity (‰)	pH	Temp. (°C)	Salinity (‰)	pH	Temp. (°C)	Salinity (‰)	pH	Temp. (°C)	Salinity (‰)	pH
T1	27.3	28.5	10.8	24.9	28.9	9.6	25.1	28.2	8.3	23.1	29.0	8.2
T2	27.1	28.6	10.6	25.0	28.8	10.2	25.2	28.6	8.2	23.4	28.8	8.2
T3	27.1	28.5	10.6	25.2	28.7	10.6	24.8	28.5	8.3	23.5	28.6	8.3
T4	26.9	28.7	10.6	25.3	28.9	10.4	25.1	28.7	8.3	23.5	28.5	8.2
T5	27.0	28.5	9.7	25.2	28.9	9.7	25.2	28.5	8.2	23.4	28.6	8.2

RESULTS AND DISCUSSION

To investigate the effects of *S. platensis* as a feed additive and probiotics on the growth of the shrimp *F. chinensis*, the shrimp were cultured on a microcosm scale for 35 days. The mean body weight and survival rate of the shrimp were surveyed, along with the water quality and changes in the phytoplankton with each treatment.

Water Quality

The water temperature, salinity, and pH in the rearing tanks during the experiment are listed in Table 2. The water temperature gradually decreased from 27.3 to 23.1°C over the time period; however, the range was within the proper growth conditions for *F. chinensis*, and quite stable for all the treatments. The salinity also remained very stable within a range of 28.2 to 29.0‰ (optimal salinity, 20–32‰ for *F. chinensis*, Jang *et al.* [14]), whereas the pH values estimated on days 7 and 14 were high at 9.6–10.8, and then decreased and remained stable at 8.2–8.3 by days 21 and 35.

As shown in Table 3, the TDP and TDN concentrations gradually decreased and remained stable, respectively, during the rearing time. The TDP concentration was high, ranging from 0.02 to 0.05 mg/l on day 7, and then decreased to 0.001–0.029 mg/l, except for 0.085 mg/l for T2, by day 14. T2 and T3 showed a temporary rapid increase and decrease in the TDP concentration, respectively, on day 14, suggesting an effect from the water quality caused by the probiotics (T2) and *Spirulina* (T3). Except for T2, the TDN concentration remained stable during the culture period, ranging from 1.11 to 1.49 mg/l. However, for T2, the TDN concentration decreased rapidly to 0.25 mg/l by day 21, which was 5 times lower than that for the other treatments, and then increased to 1.81 mg/l by day 35. It is supposed that the sudden high TDP on day 14 and low TDN on day 21 in T2 resulted from the addition of *Bacillus* spp. and *Lactobacillus* spp. (probiotics). For both the TP (total phosphorus) and TN (total nitrogen), T1 was high and T5 low, as expected, whereas T2 was low following T5 (data not shown), suggesting that the probiotic treatment without *S. platensis* was more effective in improving the water quality.

Lim *et al.* [18], who previously examined the effects of probiotics on the water quality in *F. chinensis* culture ponds, reported that the TDN concentration was initially high within a range of 3.55 to 3.65 mg/l, yet gradually decreased to 0.63–1.93 and 0.93–6.85 mg/l in a pond treated with and without probiotics, respectively. Thus, the TDN concentration in the present experiment was similar to the results of Lim *et al.* [18], although the 0.25 mg/l for T2 including probiotics was lower in comparison. As regards the TDP concentration, the present results were also lower than those reported by Lim *et al.* [18]. These different results may have been due to the flourishing phytoplankton flora and low stocking-density of *F. chinensis* in this experiment. However, the TDN and TDP in T2 still changed dramatically, suggesting the probiotics had a significant effect on the water quality. Therefore, it is expected that a long-term experiment using probiotics will produce more stable and effective results owing to the improved water quality.

The efficacy of water quality control (inorganic nitrogen concentration: ammonia, nitrite, and nitrate) using microalgae

Table 3. Means (SD) of TDP (total dissolved phosphorus) and TDN (total dissolved nitrogen) in each treatment during experiment (n=3).

Treatment	TDP (mg/l)				TDN (mg/l)			
	Day 7	Day 14	Day 21	Day 35	Day 7	Day 14	Day 21	Day 35
T1	0.039 (0.000)	0.029 (0.000)	0.008 (0.000)	0.015 (0.000)	1.345 (0.023)	1.301 (0.023)	1.318 (0.023)	1.412 (0.040)
T2	0.048 (0.004)	0.085 (0.004)	0.039 (0.000)	0.008 (0.000)	1.251 (0.040)	1.265 (0.028)	0.252 (0.040)	1.812 (0.040)
T3	0.044 (0.004)	0.001 (0.000)	0.014 (0.002)	0.008 (0.000)	1.105 (0.023)	1.292 (0.040)	1.43 (0.023)	1.492 (0.000)
T4	0.021 (0.002)	0.015 (0.000)	0.008 (0.000)	0.010 (0.002)	1.278 (0.023)	1.318 (0.023)	1.305 (0.023)	1.412 (0.000)
T5	–	0.005 (0.000)	0.008 (0.002)	0.000 (0.002)	–	1.305 (0.046)	1.292 (0.000)	1.398 (0.023)

for shrimp culture tanks has already been clearly demonstrated by Chuntapa *et al.* [7], where *S. platensis*, was used to improve the water quality, and semicontinuous harvesting shown to be an effective method for water quality control even with a high shrimp density. Therefore, in the present study, the fluctuation of the phytoplankton flora was monitored in the water column of the rearing tank.

Chlorophyll *a* and Phytoplankton Flora

As shown in Fig. 1A, the chlorophyll *a* concentration gradually increased and peaked on day 21 with a distribution of 76.5–123.6 μg/l. Yet, by day 35, there was an overall decrease within a range of 47.3–74.2 μg/l, except for T3, which showed a continued increase to 106.1 μg/l on day 35, indicating that the abundant nutrition of *S. platensis* may have affected the flourishing of the phytoplankton. The trend in the chlorophyll *a* concentration was also reflected in the standing crop of phytoplankton, although contrary to the low concentration of chlorophyll *a* on day 35, the standing crop continued to increase and peaked with a high cell density ranging from 154,000 to 971,000 cells/ml (Fig. 1B). However, this discrepancy between the chlorophyll *a* concentration and the standing crop can be explained by the fluctuation of the phytoplankton flora.

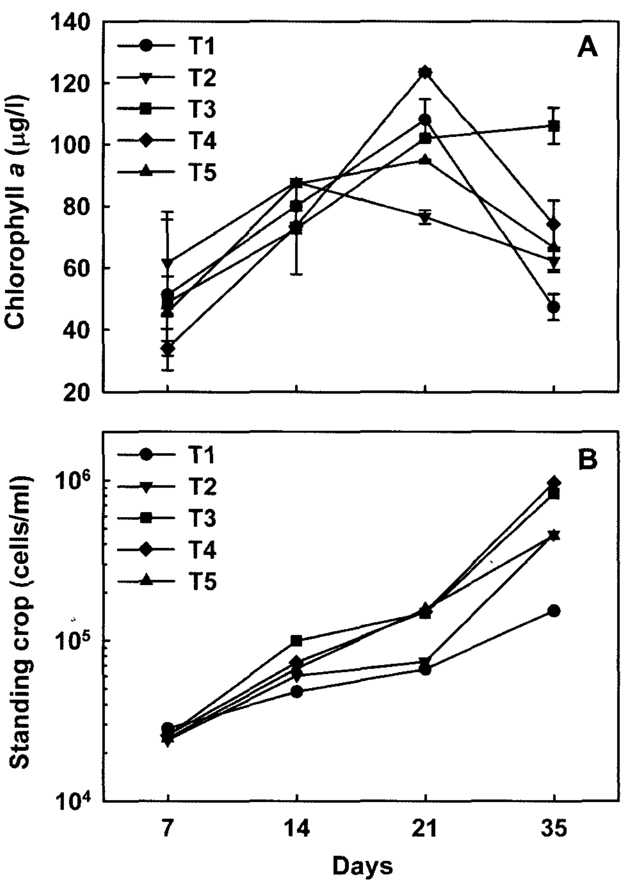

Fig. 1. Change in chlorophyll *a* (**A**) and phytoplankton standing crop (**B**) with each treatment during experiment. Error bars indicate SD.

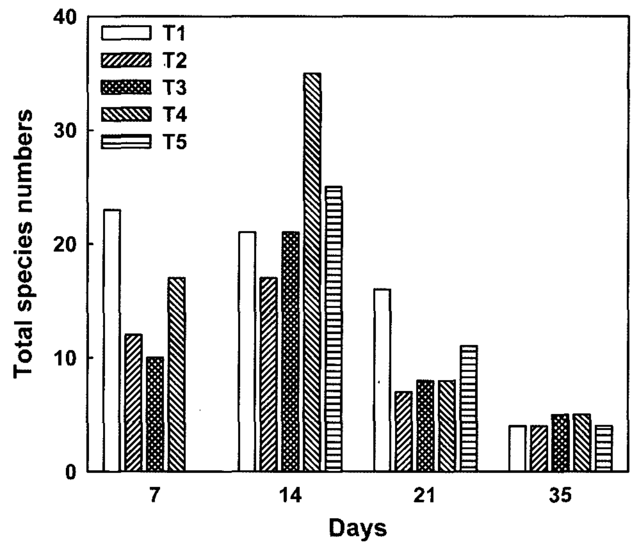

Fig. 2. Total phytoplankton species numbers for each treatment during experiment. The sample of T5 on day 7 was lost.

The phytoplankton identified in the present study was divided into 5 classes: Bacillariophyceae, Cyanophyceae, Chlorophyceae, Dinophyceae, and Euglenophyceae with an amoeboidal cell group. A total of 85 phytoplankton species were identified during the shrimp culture, with 35 species on day 7, 52 species on day 14, 45 species on day 21, and 32 species on day 35, indicating that the species diversity initially increased to a peak on day 14, and then decreased thereafter (Fig. 2). Among the treatments, high species numbers occurred with T1 and T4, T4 and T5, T1 and T5, and T3 and T4 on days 7, 14, 21, and 35, respectively, indicating that the effect of *S. platensis* (T3) and the probiotic additive (T2) on the phytoplankton diversity was not significant. However, there was a sharp increase in the phytoplankton species number in T4 on day 14, but the reasons were not clear. Members of Bacillariophyceae dominated the species numbers during the initial culture, representing 44–65%, but this gradually decreased to below 47% with the increasing dominance of cyanobacteria, which began to increase on day 14 with 20–43% and flourished to 42–60% by day 35 (Fig. 3).

In general, domination by diatoms (centric diatoms) is more favorable than domination by cyanobacteria as regards maintaining the water quality in a shrimp pond, as centric diatoms are important food items for higher consumers, do not form noxious algal blooms, and are non-toxic [4, 21, 22]. Among the diatoms, *Thalassiosira weissflogii* would be expected to support a successful shrimp culture in a still pond. The "water making" by farmers refers to brown water dominated by diatoms, which is recognized as the best water for shrimp culturing. The abundance of *T.*

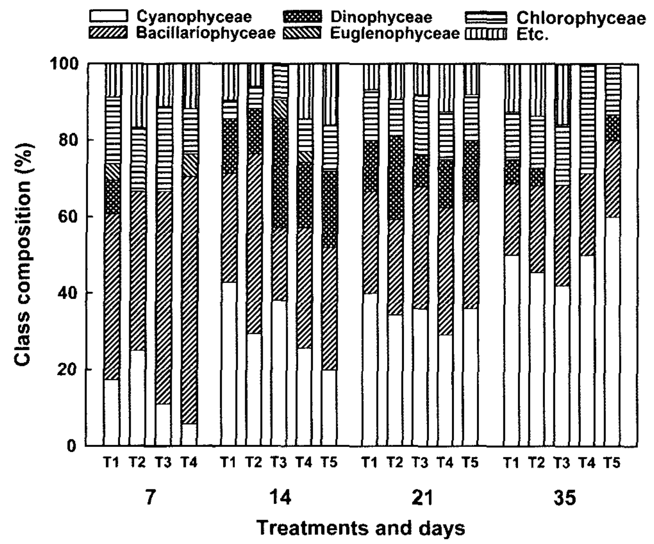

Fig. 3. Composition change (%) in phytoplankton species number for each treatment during experiment.

weissflogii was also confirmed in the shrimp culture pond near the experimental tanks. However, in the present study, although diatom species occurred, they did not dominate.

The noted succession of phytoplankton flora from Bacillariophycea to Cyanophyceae was similar to the results of Yusoff *et al.* [30], who reported that Bacillariophyceae was dominant and Cyanophyceae absent in both a probiotics-treated and control pond at the beginning of the culture period, whereas later the Bacillariophyceae decreased significantly while the Cyanophyceae increased in both ponds. However, in the present study, the phytoplankton flora succeeded from Bacillariophyceae to Cyanophyceae, regardless of the feed additives. Burford [5] previously suggested that a phytoplankton succession from Bacillariophyceae to Cyanophyceae may be related to a low silicate concentration. Therefore, in the present tank culture, since there was no change of water, a decrease in the silicate concentration may have limited the diatom growth.

The major dominant species were *Oocystella radiosa* (Chlorophyceae) and *Thalassiosira eccentrica* (Bacillariophyceae) on days 7 and 14, and *O. radiosa* and *Oscillatoria* sp. (Cyanophyceae) on days 21 and 35 (data not shown). In particular, the high standing crops of phytoplankton by day 35 were influenced by the number of filamentous *Oscillatoria* sp. and *O. radiosa* growing in a sac-like architecture. The low chlorophyll *a* concentration on day 35 was because the measurements taken from the water column that was well mixed by the aeration in the tanks did not account for the biofilms that attached to the tanks and grew densely during the experimental period, achieving a stable community with the decreasing water temperature. Therefore, this stable biofilm formation inevitably contributed to the decreasing chlorophyll *a* concentration in the rearing water column.

The biofilms have been recognized for their important role in water quality [13, 19]. Thompson *et al.* [28] previously reported that biofilms reduced the exportation of phosphorus and produced a higher output of nitrate+nitrite, instead of ammonium, with important implications as a food source for shrimp. Therefore, in the present experiment, the low TDP and TDN concentrations were likely achieved by the phytoplankton along with the well-developed biofilms, rather than by the direct effect of the feed additives. Nonetheless, further studies are needed to examine the effect of feed additives on the formation and flourishing of biofilms.

Mean Body Weight and Survival Rate of Shrimp

The mean body weight and survival rate of the *F. chinensis* cultured for 35 days with the different treatments are shown in Fig. 4. The mean body weight of the cultured shrimp ranged from 9.3 to 14.8 g. Treatment T3, including *S. platensis*, produced the highest mean body weight, which was 39% higher than that for all the other treatments ($P<0.05$). Treatment T4, including 1.5% *S. platensis* and 1.5% probiotics, produced a better result of 11.2 g compared with the control at 10.7 g ($P>0.05$). Treatment T5, which was half the basal diet with 1.5% *S. platensis*, produced the lowest mean body weight of 9.3 g. Unexpectedly, treatment T2, including only probioics, had no remarkable effect on the shrimp body weight at 10.6 g compared with the control T1 at 10.7 g. Therefore, these results suggest that the use of *S. platensis* as a feed additive significantly improved the *F. chinensis* growth. It is assumed that the balanced nutrition and improved activity towards pathogenic organisms with the addition of the feed additive *Spirulina* at a high ratio (3%) to the commercial diet produced the

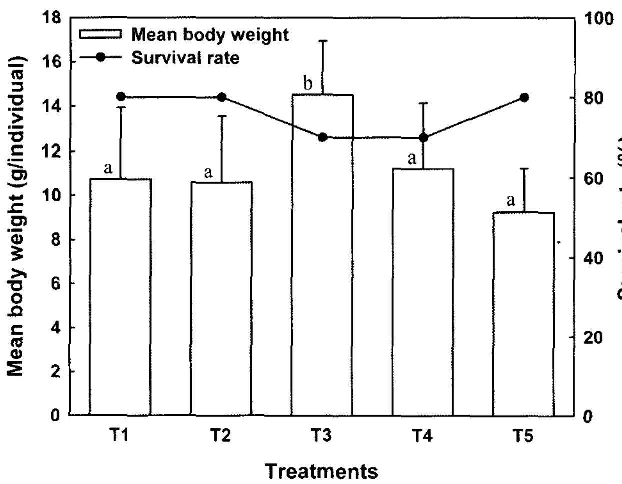

Fig. 4. Mean body weight (g) and survival rate (%) of *Fenneropenaeus chinensis*, reared in each tank, at the end of experiment. Different letters on the bar indicate significant difference between treatments ($P<0.05$).

significant body weight increase for the T3 shrimp, despite the short culture period and limited space (1 m diameter) in the culture tank. Overall, the survival rates were high for all the treatments, ranging from 70 to 80%, which was attributed to a stable phytoplankton community containing biofilms and low stocking-density of *F. chinensis* with minimal feeding. The immune modulation in shrimp caused by *Spirulina* has already been well demonstrated by Lee [16], where the granulocyte and hyaline cell activity of tiger prawns supplied with a feed containing as low as 0.1% (w/w) dry *Spirulina* was significantly enhanced, whereas shrimp fed with *Spirulina* were able to eliminate the shrimp pathogen *Vibrio parahaemolyticus* from the hemolymph in half the time compared with the control fed with a basal diet.

In a recent study, juvenile tilapia fed raw *Spirulina* exhibited a worse body weight and body length, yet better survival rate compared with those fed a commercial diet [27]. However, contrary to the study of Takeuchi *et al.* [27], the present experiment showed a remarkable body weight increase for the shrimp fed the *Spirulina* feed additive, implying that the *Spirulina* feed additive improved growth. Conversely, the probiotics were more effective in improving the water quality rather than the body weight growth of the shrimp. Accordingly, the survival rate of shrimp, which is generally very low in an aquaculture, may be improved with the use of *Spirulina* as a feed additive, while also maintaining strict control of the water quality by adding the appropriate probiotics.

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REFERENCES

1. American Public Health Association. 1992. *Standard Methods for the Examination of Water and Wastewater.* APHA, Washington DC, U.S.A.
2. Asian Shrimp Culture Council. 1995. Mass mortality caused by systemic bacilliform virus in cultured penaeid shrimp, *Penaeus monodon*, in Thailand. *Asian Shrimp News* **21:** 2–4.
3. Belay, A. 2002. The potential application of *Spirulina* (*Arthrospira*) as a nutritional and therapeutic supplement in health management. *J. Am. Nutraceut. Assoc.* **5:** 27–43.
4. Boyd, C. E. 1990. *Water Quality in Ponds for Aquaculture.* Auburn University, Auburn, AL, U.S.A.
5. Burford, M. 1997. Phytoplankton dynamics in shrimp ponds. *Aquacult. Res.* **28:** 351–360.
6. Cho, S. H., S. B. Hur, and J. Y. Jo. 2001. Effect of enriched live feeds on survival and growth rates in larval Korean rockfish, *Sebastes schlegeli* Hilgendorf. *Aquacult. Res.* **32:** 199–208.
7. Chuntapa, B., S. Powtongsook, and P. Menasveta. 2003. Water quality control using *Spirulina platensis* in shrimp culture tanks. *Aquaculture* **220:** 355–366.
8. Duncan, P. L. and P. H. Klesius. 1996. Effects of feeding *Spirulina* on specific and nonspecific immune responses of channel catfish. *J. Aquat. Anim. Health* **8:** 308–313.
9. Fuller, R. 1989. Probiotics in man and animals. *J. Appl. Bacteriol.* **66:** 365–378.
10. Gupta, A. P. 1986. Arthropod immunocytes, pp. 3–42. *In* A. P. Gupta (ed.). *Hemocytic and Humora Immunity in Arthropods.* John Wiley & Sons, New York, U.S.A.
11. Holmblad, T. and K. Soderhall. 1999. Cell adhesion molecules and antioxidative enzymes in a crustacean; possible role in immunity. *Aquaculture* **172:** 111–123.
12. Hopkins, S. J., R. D. II. Hamilton, P. A. Sandifer, C. L. Browdy, and A. D. Stoker. 1993. Effect of water exchange rate on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp ponds. *J. World Aquacult. Soc.* **24:** 304–320.
13. Jang, A. M., M. S. Bum, S. Y. Kim, Y. H. Ahn, I. S. Kim, and P. L. Bishop. 2005. Assessment of characteristics of biofilm formed on autotrophic denitrification. *J. Microbiol. Biotechnol.* **15:** 455–460.
14. Jang, I. K., M. W. Park, D. S. Jeong, J. H. Song, Y. S. Choi, H. J. Lim, and J. H. Kim. 2000. *Shrimp Farming and Disease Control*, pp. 127. Youngin Press, Seoul (in Korean).
15. Klein, J. 1989. Are invertebrates capable of anticipatory immune responses? *Scand. J. Immunol.* **29:** 499–505.
16. Lee, K. 1999. *Spirulina* and immunological activity of cultured prawn. *Book of Abstracts.* Second Asian Pacific Phycological Forum. M-25.
17. Lee, Y.-K., P.-F. Chew, B.-S. Soh, and L.-Y. Tham. 2003. Enhancing phagocytic activity of hemocytes and disease resistance in the prawn *Penaeus merguiensis* by feeding *Spirulina platensis*. *J. Appl. Phycol.* **15:** 279–287.
18. Lim, H. J., J. H. Park, and I. K. Jang. 2004. Effect of probiotics on water quality in shrimp (*Fenneropenaeus chinensis*) ponds. *J. Kor. Fish. Soc.* **37:** 91–97.
19. Na, B.-K., B.-I. Sang, D.-W. Park, and H.-P. Doo. 2005. Influence of electric potential on structure and function of biofilm in wastewater treatment reactor: Bacterial oxidation of organic carbons coupled to bacterial denitrification. *J. Microbiol. Biotechnol.* **15:** 1221–1228.
20. Nandeesha, M. C., B. Gangadhar, T. J. Varghese, and P. Keshavanath. 1998. Effect of feeding *Spirulina platensis* on the growth proximate composition and organoleptic quality of common carp, *Cyprinus carpio* L. *Aquacult. Res.* **29:** 305–312.
21. Officer, C. B. and J. H. Ryther. 1980. The possible importance of silicon in marine eutrophication. *Mar. Ecol. Prog. Series* **3:** 83–91.
22. Paerl, H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine and inland waters. *Limnol. Oceanogr.* **33:** 823–847.

23. Porubcan, R. S. 1991. Reduction of ammonia nitrogen and nitrite in tanks of *Penaeus monodon* using floating biofilters containing processed diatomaceous earth media pre-inoculated with nitrifying bacteria. Program and Abstracts of the 22nd Annual Conference and Exposition, 16-20 June 1991, San Juan, Puerto Rico. World Aquaculture Society.
24. Porubcan, R. S. 1991. Reduction in chemical oxygen demand and improvement in *Penaeus monodon* yield in ponds inoculated with aerobic *Bacillus* bacteria. Program and Abstracts of the 22nd Annual Conference and Exposition, 16–20 June 1991, San Juan, Puerto Rico. World Aquaculture Society.
25. Rengpipat, S., W. Phianphak, S. Piyatiratitivorakul, and P. Menasveta. 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture* **167:** 301–313.
26. Strickland, J. D. H. and T. R. Parson. 1968. *A Practical Handbook of Seawater Analysis*, pp. 1–311. Fisheries Research Board of Canada, Ottawa.
27. Takeuchi, T., L. Jun, G. Yoshizaki, and S. Satoh. 2002. Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw *Spirulina*. *Fish. Sci.* **68:** 34–40.
28. Thompson, F. L., P. C. Abreu, and W. Wasielesky. 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. *Aquaculture* **203:** 263–278.
29. Thornqvist, P.-O. and K. Soderhall. 1997. Crustacean immune reactions, a short review, pp. 203–217. *In* T. W. Flegel, and I. H. Macrae (eds.), *Disease in Asian Aquaculture III*. Fish Health Section, Asian Fisheries Society, Manila, The Philippines.
30. Yusoff, F. M., M. S. Zubaidah, H. B. Matias, and T. S. Kwan. 2002. Phytoplankton succession in intensive marine shrimp culture ponds treated with a commercial bacterial product. *Aquacult. Res.* **33:** 269–278.
31. Ziemann, D. A., W. A. Walsh, E. G. Saphore, and K. Fulton-Bennett. 1992. A survey of water quality characteristics of effluent from Hawaiian aquaculture facilities. *J. World Aquacult. Soc.* **23:** 180–191.