CHAPTER 5

DISCUSSION

5.1 Cultivation of *Spirulina platensis*

5.1.1 Laboratory cultures of *S. platensis*

The optimum N : P ratio for the cultivation of *S. platensis* in different media including modified Zm as control group, Kw (kitchen wastewater**)** and Sw (oil-extracted soybean meal) media, together with its biomass production (dry weight) were measured and compared among one another. The results were shown in (Appendix B. Table 8) and Figure 22. The results indicated that the initial N : P ratio of modified Zm was 6.9 : 1 and changed to 1.2 : 1 after 15–day cultivation. The N : P ratio of Kw and Sw media ranged between 6:1-6.2 : 1 and 8.2 : 1-8.3 :1, and changed to 0.4 : 1-0.5 : 1 and 6.92 : 1- 6.94 : 1, respectively, after 15-day. This is because *S. platensis* cultivation can be considered as a promising approach for nitrogen and phosphorus removal from wastewater source which, as result, the decreased N : P ratio after 15 days (Chuntapa *et al*., 2003). The cultivation of *S. platensis* in laboratory, biomass production of the cultures in 5%Sw (0.90 g L^{-1} and N: P = 6 : 1) was clearly higher than the 10-day cultures in 10-100% Kw media and 10-100%Sw media (Appendix B. Table 8). Similarly, the optimum nitrogen concentrations for algal growth is in the range 1.3-6.5 mg L^{-1}, while the optimum N : P ratio for *S. platensis* culture is 6-8 : 1 (Reynold, 1986) and nitrogen was regarded to limit primary production in freshwater aquatic ecosystems. Because primary production sets the rate of community metabolism, one speaks of these systems as N-limited (Rhyther and Dunstan, 1971).

This work showed dramatic improvements in water quality, sufficient to meet the standards for discharge, and yet produced significant amounts of useful algal material. Results, the cultivation of *S. platensis* in modified Zm, Kw and Sw, the optimum N: P ratio was 6-8 : 1. Similar to those in former studies using the wastewater arising from the production of sago starch had a high carbon/ nitrogen (C : N) ratio and effluent sources with an average C : N : P ratio of 24 : 6.14 : 1 (Siew-Moi *et al*., 2000).

The biomass of *S. platensis* was also accounted for and evaluated as a protein source for fish. It was indicated that this could support the production of large amounts of *S. platensis* in the experimental ponds with an average specific growth rate of 0.51 g L^{-1} day^{-1}. This performance is comparable to the 0.54 g L^{-1} day^{-1} of *S. platensis* cultivated in an inorganic medium (Siew-Moi *et al*., 2000). The initial pH values in the experiment ranged from 6.84 to 8.99 after 15 days cultivation, however the pH raised up ranged 8.13 - 9.48 (Appendix B. Table 8) consistent with the usual behavior of blue green algae cultures. In the former commercial growth of *S. platensis*, the media employed had high pH range (8.5-12) which they are particularly selective for this organism (algae), an important factor in preventing contamination of the reactor by bacteria, algae and protozoa (Walach *et al*., 1987).

5.1.2 Outdoor mass culture of *S. platensis* in experimental tanks

Cultivation of *S. platensis* in modified Zm, 100% Kw, 90% Kw, 10% Sw and 5%Sw media were carried out (using the selected cultures from the treatments of

S. platensis in laboratory). The cultures biomass productions were finally measured in dry weight and compared among different media used, as well as other variables including production variable cost, β-carotene, C-phycocyanin and γ- linoleic acid, nutritional values and phytoremediation.

Cultivation of *S. platensis* using different culturing media: The biomass production (dry weight; g L^{-1}) of *S. platensis* in 5%Sw medium, which was 0.90 g L^{-1} with the optimum N : P ratio of 6.3 : 1, was actually higher than those in 10%Sw (0.85 g L^{-1}; N : P ratio 6.4 : 1), modified Zm (0.84 g L^{-1}; N : P ratio 6.6 : 1), 100%Kw (0.82 g L^{-1}; N : P ratio 3.7 : 1) and 90%Kw media (0.74 g L^{-1}; N : P ratio 3.7 : 1) as show in (Appendix B. Table 9) and Figure 23. These results are similar to those in former studies of the optimum concentrations of nitrogen for algal growth where the values ranged between 1.3-6.5 mg L^{-1} and the optimum N : P ratio of between 6-8 : 1 (Reynolds, 1986). The highest value of biomass production of *S. platensis* in present study is actually higher than cultivation of *S. platensis* in wastewater originated from the digested pig waste where the biomass production was 0.77 g L^{-1} (Marquez, 1995). In addition, the highest biomass production in this study was also higher than that of *S. platensis* in 50% effluent of pig manure biogas digester (0.32 g L^{-1}) (Promya and Traichaiyaporn., 2003).

The β-carotene, C-phycocyanin and γ- linoleic acid: From Appendix B. Table 10 and Figure 24, the β-carotene and C-phycocyanin from *S. platensis* cultured in 10%Sw medium, which were 0.42 mg g^{-1} and 23.32 mg g^{-1}, respectively, were significantly higher than those in modified Zm, 100%Kw, 90%Kw and 5%Sw media with $p<0.05$. The present study utilized a modified Zm which substantially fewer nutrients compared to the standard Zm so substantially lower levels of β-carotene and

C-phycocyanin were anticipated. *S. platensis* cultured outdoors, using standard Zm, had the β-carotene and C-phycocyanin levels of 1.5 mg g^{-1} and 60.70 mg g^{-1}, respectively (Carlos *et al*, 2003). Although fewer nutrients were utilized in the modified Zm, they were sufficient to maintain the *S. platensis* culture. Additionally, with fewer nutrients, the production cost using the modified Zm was lower than that of the standard Zm.

The γ- linoleic acid (GLA) from *S. platensis* cultured in modified Zm, which was 0.302 mg g^{-1}, was significantly higher than those in 100%Kw, 90%Kw, 10%Sw and 5%Sw media. The present study was applied with the same evaluation of the effect of low light flux and nitrogen deficiency on growth and chemical composition of *Spirulina* sp. (straight filaments strain, SF) in batch cultures utilizing a complex medium containing sea-water supplemented with anaerobic effluents from digested pig waste. As the results, the palmitoleic acid percentage of total fatty acids was significantly higher ($p<0.05$) at a higher light intensity of 144 μmol photon m^{-2} s^{-1} where a high level of γ- linolenic acid (GLA) observed by 28.13% (Eugenia *et al*., 2001; Martin *et al*., 2005).

Variable cost of *S. platensis* production: Variable cost of *S. platensis* culture was calculated on a basis of Thai-baht/kg as dry weight. The culture in 5% Sw medium yielded considerable cost savings of 23.82% compared to that in modified Zm (control group). The variable cost of the cultures in 100%Kw and 10%Sw media were 10.9% and 8.08%, respectively, compared to that in modified Zm, while the value in 90%Kw was only 2.6% comparatively. This indicated that the production variable cost of *S. platensis* in the present study yielded the most considerable cost savings in 5%Sw medium (Appendix B. Table 11).

Nutritional values: From Appendix B. Table 11 and Figure 25, percent protein content in *S. platensis* cultured in modified Zm was nearly 60% higher than those in 100%Kw, 90%Kw, 10%Sw and 5%Sw were 35.86%, 32.27%, 41.49% and 20.55% (dry weight), respectively. Because modified Zm were utilized chemical nutrients in *S. platensis* culture. However, the fat content in *S. platensis* cultured in 100%Kw and 90%Kw media were 3.13% and 2.82% (dry weight), which were higher than that in modified Zm. Fiber contents of *S. platensis* in the cultures of 100%Kw, 90%Kw, 10%Sw and 5%Sw were 10.65%, 9.58%, 8.04% and 4.70% (dry weight), respectively were actually higher than that in modified Zm, probably because the kitchen wastewater was rich in carbon sources (carbohydrate and fats/oils), and the oil-extracted soybean fermented water has higher fats/oil content compared to the sole carbon source in modified Zm, the sodium bicarbonate which further study is needed. The inverse relationship between moisture content of the cultures in 100%Kw, 90%Kw, 10%Sw and 5%Sw were 7.72%, 6.95%, 8.01% and 8.17% (dry weight), respectively lower than that in modified Zm, and the ash content of the cultures in 100%Kw, 90%Kw, 10%Sw and 5%Sw were 27.94%, 25.20%, 27.50% and 22.60% (dry weight), respectively, higher than that in modified Zm, suggested a high inorganic mineral content in algae grown in kitchen waste and oil-extracted soybean fermented water.

The present study was applied with the same nutritional values of *S. platensis* dried by various methods: tray dried at 30 ^{o}C, 40 ^{o}C, 50 ^{o}C, sun dried and spray dried, were compared. The average nutritional values found were 47.45 –54.66% of crude protein, 1.01–2.43% fat, 26.68–40.78% carbohydrate, 3.93-14.84% moisture, 3.60–4.95% ash, 2.09–3.09% fiber and 264.23–518.54 μg g^{-1} carotenoid contents. Various drying methods produced significantly different ($p<0.05$) nutritional values left in the algae. Tray-dried and sun-dried *S. platensis* gave the highest protein and carbohydrate contents, respectively. On the other hand spray-dried *S. platensis* had the highest fat, moisture, ash, and carotenoid contents (Promya and Traichaiyapore, 2001).

The consideration was the protein content (41.49% dry weight), the β-carotene (0.42 mgL^{-1} dry weight) and C-phycocyanin (23.32 mg L^{-1} dry weight) of the culture in 10%Sw are actually higher than those in other cultures in all experimental media (Appendix B. Table 10 and 11). The biomass of *S. platensis* was also accounted and evaluated as a protein source for fish, especially *Oreochromis* sp. (Tubtim tilapia) among the possible organic nitrogen sources from wastewater ensured high nutrients, biomass production and nutritional values.

Phytoremediation of wastewater by *S. platensis*: After completing the15-day experimental period, it was found that the physical and chemical parameters of the used wastewater had changed significantly (Appendix B. Table 12 and 13). The values of such parameters in all culturing media indicated the increases in pH during the experimental period, consistent with the usual behavior of blue green algae cultures. In the commercial growth of *S. platensis*, the media employed have high pH values (9.5-12) which is particularly selective for this organism as an important factor in preventing contamination of the reactor by bacteria, algae and protozoa (Walach *et al*., 1987)

Large increases in dissolved oxygen (DO) values occurred in both preparations of kitchen wastewater and oil-extracted soybean fermented water media as a result of aeration. In general, the optimum level of DO for algae cultures in the water is about > 5 mg L^{-1} (Marquez, 1995) so the final solutions under aeration had adequate levels of 6.02-7.20 mg L^{-1}.

BOD values for the cultures in 100%Kw, 90%Kw, 10%Sw and 5%Sw media decreased by 71.08 to 96.28%. However, the chemical composition of the culture in modified Zm resulted in a low BOD initially since it contains no significant levels of biodegradable organic compounds such as proteins, carbohydrates or fats. The present study, using kitchen wastewater and oil-extracted soybean fermented water as the culturing media, indicated that a larger percentage decrease in the BOD values occurred compared to the decreasing BOD percentage of 79% from the cultivation of *S. platensis* in the 40% dormitory effluent from Maejo University, Chiang Mai, Thailand (Promya and Traichaiyaporn., 2005a).

From the cultures of *S. platensis* in 100%Kw, 90%Kw, 10%Sw and 5%Sw media, the COD content decreased dramatically at 72.6, 73, 94 and 96%, respectively, but somewhat less than with cultivation of *S. platensis* in wastewater from the production of sago starch as reported by Canezares (1993), where COD levels decreased by 98.00%, and in the cultivation of *S. platensis* in 30% effluents from pig manure biogas digester reported by Promya and Traichaiyaporn (2003) where COD decreased by about 97% using different waster sources.

Improvement in water quality is often assessed by the ability of processes to reduce nutrient levels, especially nitrogen and phosphorous. Cultivating algae in water with high nutrient levels can dramatically reduce nitrogen and phosphorous levels and, at the same time, produce a useful product, such as algae biomass for animal feed. Kitchen wastewater and oil-extracted soybean fermented water generally have high levels of nutrients and large quantities of these effluents are normally produced daily at the commercial and residential sites so it is especially noteworthy that the experiments described in this work showed dramatic improvements in water quality, sufficient to meet the standards for discharge, and yet produced significant amounts of useful algal material.

Total phosphorous (TP) levels in all experimental media including modified Zm, 100%Kw, 90%Kw, 10%Sw and 5%Sw media were initially high, ranging between 3.83-9.92 mg L^{-1}, and had decreased down to percentages of 50, 50.4, 50.4, 74 and 74%, respectively. These are similar to such values in other studies where 54-75% phosphorous reductions was achieved (Martinez *et al*., 2000; Siew-Moi *et al*., 2000; Gonzales *et al*., 1997 and An *et al*., 2003)

Ammonia nitrogen (NH_3-N) was not added to the culture in modified Zm so measured levels were near the detection limit. However, there were significant levels of NH_3-N initially in the kitchen wastewater and oil-extracted soybean fermented water resulting in the values of initial ammonia nitrogen of 7.03, 5.75, 12.08 and 9.37 mg L^{-1} in the cultures in 100%Kw, 90%Kw, 10%Sw and 5%Sw, respectively. After 15 days of cultivation, the levels of ammonia nitrogen in the cultures in 100%Kw, 90%Kw, 10%Sw and 5%Sw had dropped dramatically by 98.0, 97.8, 97.66 and 98.5 %, respectively, to the levels within the allowable discharge limits for NH_3-N (<1.1 mg L^{-1}) (Pollution Control Department., 1994).

Total Kjeldahl nitrogen (TKN) is the sum of organic nitrogen and ammonia nitrogen (Stumm and Morgan., 1996). Since the cultures in modified Zm contained no added ammonia or organic nitrogen, the initial and final levels are very low, 0.07 and 0.05 mg L^{-1}, respectively). On the other hand, the kitchen wastewater and oil-extracted soybean fermented water contained significant levels of both organic and ammonia nitrogen, so the initial TKN levels are high as 22.56, 20.3, 45.46 and 24.34 mg L^{-1} for the cultures in 100%Kw, 90%Kw, 10%Sw and 5%Sw media, respectively. Culturing *S. platensis* in kitchen wastewater and oil-extracted soybean fermented water resulted in 97% decreases in TKN for both the cultures in 100%Kw and 90%Kw media, while 94% decreases in TKN was observed in both the cultures in 10%Sw and 5%Sw media. The resulting TKN values of the water after 15-day cultivation acceptable was within the discharge limit of <100 mg L^{-1} (Pollution Control Department., 1994). Since organic nitrogen (ON) is derived from TKN by subtracting the NH_3-N value, similar decrease of nearly 97% in kitchen wastewater was observed.

Nitrate nitrogen (NO_3-N) was the sole source of nitrogen for the culture in modified Zm so the initial level is very high at 30.73 mg L^{-1}. Cultivation of *S. platensis* had reduced this level by 94.2%. The lower starting levels in the cultures in 100%Kw, 90%Kw, 10%Sw and 5%Sw media of 03.8, 3.47, 31.43 and 11.47 mg L^{-1}, were also dramatically reduced by 99.2, 99.2, 99.77 and 99.20 %, respectively. Again, in all experimental media, after the cultivation period, the nitrate nitrogen levels were within the discharge limit (< 0.5 mg L^{-1}) (Pollution Control Department., 1994). Nitrite nitrogen (NO_2-N) levels in all cultures in all experimental media after 15-day cultivation were very low, between 0.003 – 0.07 mg L^{-1}, as anticipated, since neither modified Zm nor kitchen wastewater and oil-extracted soybean fermented water had an identifiable source of this nutrient.

Total oxidized nitrogen (TON) is the sum of NO_3-N and NO_2-N. This quantity is dominated by the effects of NO_3-N <0.5 mg L^{-1} (Pollution Control Department., 1994). Total nitrogen (TN) is the sum of TKN and TON and the resulting values are summarized in Table 10 and 11. 94% to 97% reductions of TN were achieved in all cultures of all experimental media after 15 days of cultivation of *S. platensis*.

5.1.3 Mass culture of *S. platensis* in cement raceway ponds and earthen raceway ponds

The biomass production, water quality and nutritional values of *S. platensis* cultured in cement raceway ponds and earthen raceway ponds for 10 days were not significantly different. The water quality in both cement raceway ponds and earthen raceway ponds were derived from using the same oil-extracted soybean fermented water as effluent source of culturing medium (10%Sw medium). The biomass production value (dry weight) of 0.85 g L^{-1}, production variable cost (dry weight) of 221.58 baht kg^{-1} and protein content (dry weight) of 40.82% for the cultures in 10%Sw medium in raceway cement ponds were compared to be no different to such values in raceway earthen ponds of 0.80 g L^{-1}, 240.68 baht kg^{-1} and 39.40 %, respectively. The biomass of *S. platensis* was not the bio-accumulate of heavy metals or pesticides because *S. platensis* was cultured in Kw and Sw media. The biomass of *S. platensis* was also evaluated as a protein source for fish, especially *Oreochromis* sp. (Tubtim tilapia). Young tilapias are easily weaned and grow fast to market size when fed with formulated diets. Fast growth rates are common when fish are fed with foodstuffs containing levels of 20-30% protein (Mahajan and Kamat, 1995).

5.2 Tuptim tilapia culture

5.2.1 Nursing larval Tuptim tilapia in the earthen pond using raw *S. platensis*

Growth performance

Although, nursing of larval Tuptim tilapia in the earthen pond, using raw *S. platensis* form different diet-treatments including 5% commercial diets (5%CD) as control, 1% raw *S. platensis* (1%RS), 3% raw *S. platensis* (3%RS) and 5% raw *S. platensis* (5%RS), the present study resulted as 5%CD had enhanced the highest average weight, weight gain and specific growth in the larval fish during the first 60 days of the experiment (day0-60), however, during day-60 and the completion at day-90, the resulted performances of all the feeding formulas were not statistically different. This indicated that the raw *S. platensis* was a promising partial in feeding formula for nursing larval Tuptim tilapia (Figure 29 and 30). The same experimental effect of *S. platensis* on pigmentation, growth, survival rate of ranchu fish and red tilapia fish were prior studied and it was suggested that *S . platensis* can be a source of carotenoid pigment for color improvement of ranchu fish and red tilapia fish (Lu and Takeuchi., 2003). The present study has also shown the same result with the development of native *S. platensis* and *Cladophora* sp. for nursing of fancy carps, where the result indicated that, using raw *S. platensis* as feed addition, the fish had significantly higher specific growth rate, percentage weight gain and survival rate compared to powder feed and dry *Cladophora* sp. (Promya and Hongvityakorn, 2003).

The estimation of larval Tuptim tilapia immunity with the treatment of using raw *S. platensis* as feed

The best immunity as unit of lysozyme activity assay of the nursing larval Tuptim tilapia was achieved in the fish from 5%RS, where the value is 8.11 units mL^{-1}, and the fish from 3%RS, where the value is 8.71 units mL^{-1}, after being cultivated for 90 days. The red blood cell and white blood cell count of the nursing larval Tuptim tilapia from 5%RS were 2.88 x 10^{6} cell μL^{-1} and 109 x 10^{3} cell μL^{-1}, respectively. These values were significantly higher than those obtained in the fish from 3%RS, 1%RS and 5%CD (appendix B Table 16 and Figure 31). The present study indicated the same amount of red blood cell count to the hematology reference intervals for hybrid tilapia of between 1.91 to 2.83 x 10^{6} cell μL^{-1} cultured in high-density production systems (Terry *et al*., 2000).

The C-phycocyanin in *S. platensis* has been used mainly as a food pigment. However, small quantities were included as biochemical tracers in immunoassays (Vonshak, 1997). The present study of *S. platensis* had shown the same effects to the commercially available algae *Dunaliella* extract on growth, immune functions and disease resistance which were determined in the experiment of black tiger shrimp (*Penaeus monodon*) where the total carotenoid level was highest in shrimp fed with 200–300 mg of the *Dunaliella* extract. The *Dunaliella* extract showed beneficial effects as a shrimp feed supplement (Kidchakan *et al*., 2005).

Analyses for physico-chemical water quality in earthen pond nursing larval Tuptim tilapia

The result of analyses for physic-chemical quality of the effluent from the earthen ponds nursing larval Tuptim tilapia are as shown in (Appendix B. Table 17) and Figure 32. The pH in the earthen ponds nursing larval Tuptim tilapia ranged from 7.17 to 8.23 at 90 days cultivation. Three values of highest alkalinity in the nursing larval Tuptim tilapia earthen pond was achieved in the effluents from 5%RS (109.70 mg L^{-1}), 3%RS (107.60 mg L^{-1}) and 1%RS (112.75 mg L^{-1}) which are higher than that in the effluent from 5%CD (73.23 mg L^{-1}) at 90 days cultivation. The TDS and conductivity in the nursing larval Tuptim tilapia earthen pond with 5%RS was significantly higher than those values with 3%RS, 1%RS and 5%CD at 90 days cultivation. The DO value in the nursing larval Tuptim tilapia earthen pond with 5%CD ranged from 5.70 to 7.97 mg L^{-1} which are higher than those with 1%RS, 3%RS and 5%RS at 90 days cultivation. The PO_4-P in the nursing larval Tuptim tilapia earth pond with 5%RS, 3%RS and 1%RS ranged from 0.004 to 0.005 mg L^{-1}, was significantly higher than that with 5%CD at 90 days cultivation. The NH_3-N in the nursing larval Tuptim tilapia earth pond with 5%RS, where the values ranged from 0.014 to 0.017 mg L^{-1}, was significantly higher than those with 3%RS, 1% RS and 5%CD at 90 days cultivation.

The results indicated that, pH, alkalinity, TDS, conductivity and nutrients had increased resulted from using raw *S. platensis* as feed. In the commercial growth of *S. platensis*, the media employed have high pH values ranged between 8.5 to 12 (Walach *et al*, 1987). Some physico-chemical values found in the present study were in the same ranges with the values found in former study. Those values included the optimum level of water temperature of between 28 to 32 °C, pH of between 6.0 to 8.5, DO of above 3.0 mg L^{-1}, alkalinity of between 50 to 700 mg L^{-1}, and Total ammonia nitrogen of less than 1 mg L^{-1} (Tave, 1990).

5.2.2 Culturing male and female juvenile Tuptim tilapia in the earthen pond

Growth performance of male juveniles Tuptim tilapia

The result of nursing male juvenile Tuptim tilapia with four different diet-treatments, including commercial diets with 0% raw *S. platensis* (0%RS), 55% rice polish plus 45% raw *S. platensis* (45%RS), 50% rice polish plus 50% raw *S. platensis* (50%RS) and 45% rice polish plus 55% raw *S. platensis* (55%RS), indicated no different, where the experimented male juvenile Tuptim tilapia fed with 55%RS and 50%RS had shown the highest protein efficiency ratio (Figure 33 and

34). The results from present study were similar to those in former studies with hybrid catfish (*Clarias macrocephalus* x *Clarias gariepinus* (Burchell)) fed with 10% dry weight *S. platensis* which achieved the best performance on weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and development of antibody levels against bacteria *Aeromonas hydrophila*. The total carotenoid content in fish flesh increased with the level of *S. platensis* supplemented (Phromkunthong and Pipattanawattanakul, 2005).

Growth performance of female juveniles Tuptim tilapia

The result of nursing female juvenile Tuptim tilapia with the same four different diet-treatments, including 0%RS, 45%RS, 50%RS and 55%RS indicated the highest average weight, weight gain and specific growth with 0%RS and 55%RS, while the highest protein efficiency ratio in female juvenile Tuptim were found in the fish fed with 55%RS and 50%RS (Figure 35 and 36). The results of the present study on growth rate and feed conversion ratio were also similar to those in other studies of *Probarbus jullieni* where the fish fed with 25% *S. platensis* (dry weight) had better growth rate and feed conversion ratio than the control with 0% *S. platensis* (Nandeesha *et al*., 1998). In addition, *S. platensis* has no cellulose which made it easy to be digested and the amino acid content is similar to that recommended for human or animal consumption by the international Food and Agriculture Organization (Richmond, 1998).

Estimation of juvenile Tuptim tilapia immunity

From (Appendix B. Table 22) and Figure 37, the best immunity as unit of lysozyme activity assay of the cultured Tuptim tilapia of between 8.11 to 16.98 units mL^{-1} were achieved in the fish from the culture with 55%RS at 150 days cultivation. The red blood cell count of between 2.70 to 3.56 x 10^{6} cell μL^{-1} and the white blood cell count of between 102.00 to 165.33 x 10^{3} cell μL^{-1} achieved from the culturing juveniles Tuptim tilapia in the culture with 55%RS was significantly higher than those in the cultures with 50%RS, 45%RS and 0%RS at 150 days cultivation. The present study red blood cell and white blood cell count are higher than hematology reference intervals for hybrid tilapia where RBC count was between 1.91 to 2.83 x 10^{6} cell μL^{-1} and WBC count was between 21 to 154 x 10^{3} cell μL^{-1} cultured in high- density production systems (Terry *et al*., 2000). The results from present study are similar to scientific studies with mice, hamsters, chicken, turkey, cat and fish where *S. platensis* consistently improves immune system function. Medical scientists found that *S. platensis* was not only stimulates the immune system, but it actually enhanced the body's ability to generate new blood cells. In this context, it was recently described that the antioxidant properties of a natural extract obtained from blue–green alga in protecting human erythrocytes and plasma samples against oxidative damage *in vitro* (Lissi *et al*., 2000).

The β-carotene, C-phycocyanin, γ- linoleic acid of feed, flesh and eggs in culturing juvenile Tuptim tilapia

From (Appendix B. Table 23) and Figure 38 – 40, the β-carotene from feed of juvenile Tuptim tilapia in the culture with 55%RS (0.17 mg g^{-1}) was significantly higher than those in the cultures with 50%RS, 45%RS and 0%RS. The β-carotene from flesh of juvenile Tuptim tilapia in the cultures with 55%RS (0.10 mg g^{-1}) and 50%RS (0.09 mg g^{-1}) were significantly higher than those in the cultures with 45%RS and 0%RS. However, the β-carotene from eggs of juvenile Tuptim tilapia has no significant difference. The results from the present study were similar to the effects of *S. platensis* supplement assessed for aquatic animal growth, pigment in skin and flesh, egg production, egg quality, fertility, hatchability and highly resistant disease (Nakano *et al*., 2003). Another former study of Rainbow trout, using *S. platensis* as feed addition, has also found that, after 48 hours of the experiment, the analysis β-carotene, lutein and zeaxanthin were found in the treated fish at higher levels than in the control fish group with no *S. platensis* added to feed (Latscha, 1991).

The C-phycocyanin from feed, flesh and of eggs juvenile Tuptim tilapia in the cultures with 55%RS were 14.02, 6.77 and 2.68 mg g^{-1}, respectively, which were significantly higher than those in the cultures with 50%RS, 45%RS and 0%RS. The same as the present study, it was explained that the C-phycocyanin has been used mainly as a feed pigment, however, small quantity were included as biochemical tracers in immunoassays due to their fluorescent properties (Vonshak, 1997).

The γ- linoleic acid from of feed and eggs juvenile Tuptim tilapia in the culture with 55%RS were 0.11 and 0.12 mg g^{-1}, while in the culture with 50%RS were 0.10 and 0.14 mg g^{-1}, which were significantly higher than those in the cultures with 45%RS and 0%RS. However, the γ- linoleic acid from flesh of juvenile Tuptim tilapia was not different. The results from the present study indicated that the fish in the group fed with raw *S. platensis* contain more γ-linolenic acid (GLA) compared to the fish fed without *S. platensis*. It was also concluded that tilapia fed solely on raw *S. platensis* could maintain normal reproduction throughout three generations (Lu *et al*., 2003).

Production variable cost and nutritional value in feed, flesh and eggs of juvenile Tuptim tilapia (percentage dry weight)

From (Appendix B. Table 24, 25) and Figure 41-46, total production variable cost was calculated on a unit of baht/kg of fish. The production variable cost of juvenile Tuptim tilapia from all experiments were non-significantly different. The crude protein of feed for juvenile Tuptim tilapia in all experiments were adjusted to the same level. The crude protein from flesh and eggs of juveniles Tuptim tilapia in the cultures with 55%RS were 25.97 and 37.18%, while those in the culture with 50%RS were 23.23 and 36.33%, which were significantly higher than those in the culture with 45%RS and 0%RS. *S. platensis* is a rich source of protein and also contains chlorophyll, β-carotene, C-phycocyanin, vitamin and mineral for healthy human food and animal feed (Switzer,1982; Venkataraman, 1983). The results from the present study were similar to the former studies where the crude protein analyses of flesh and eggs in experimented tilapia fish indicated the values of about 17.5- 19.4 % and 38-39% (Chien *et al*., 2006 ; Abdel *et al*., 2005).

Carbohydrate from juvenile Tuptim tilapia feed in the culture with 50%RS (49.75%) was significantly higher than those in the culture with 55%RS, 45%RS and 0%RS. Carbohydrate from flesh of juvenile Tuptim tilapia in the culture with all experiments were not significantly different. Carbohydrate from eggs of juvenile Tuptim tilapia in the culture with 55%RS was 5.32%, which were significantly higher than those in the cultures with 50%RS, 45%RS and 0%RS. The fat from feed of juvenile Tuptim tilapia with 0%RS (6.10%) was significantly higher than 55%RS, 45%RS and 50%RS, respectively. The fat from flesh of juvenile Tuptim tilapia in the culture with 45%RS (5.84%) was significantly higher than those in the cultures with 50%RS, 0%RS and 55%RS. The fat from eggs of juvenile Tuptim tilapia in the culture with 0%RS (7.32%), 45%RS (6.29%) and 50%RS (5.44%) were significantly higher than those in the cultures with 45% RS. The results from the present study were similar to a former study where the crude fat of experimented fish flesh indicated the values of about 3.8 - 4.4 % (Chien *et al*., 2006).

The fiber from feed of juvenile Tuptim tilapia in the culture with 0%RS was 3.60%, which was significantly higher than those in the cultures with 55%RS, 45%RS and 50%RS. The fiber from flesh and eggs juvenile Tuptim tilapia in the culture with all experiments were not significantly different. The results from the present study were lower than the former study where the crude fiber from eggs and flesh of tilapia fish indicated the values of about 5.3-7.9% dry weight (Abdel *et al*., 2005).

The moisture from feed of juvenile Tubtim tilapia in the culture with 45%RS (10.54%) was significantly higher than those in the cultures with 55%RS, 50%RS and 0%RS. The moisture from flesh and eggs of juvenile Tuptim tilapia with all experiments were not significantly different. The results from the present study were similar to the former studies where the moisture of experimented fish flesh and eggs indicated the values of about 74.4 - 76.6 % wet weight (Chien *et al*., 2006) and 9.8-11.6% dry weight (Osan *et al*., 2007).

The ash from feed of juvenile Tuptim tilapia in the culture with 0%RS was 15.11%, which was significantly higher than those in the cultures with 55% RS, 50% RS and 45% RS. The ash from flesh and eggs of juvenile Tubtim tilapia in the culture with all experiments were not significantly different. The results from the present study were lower than to the former study where the total ash of flesh and egg tilapia indicated the values of about 5-10% and 12.9-14.4% dry weight (Chien *et al.*, 2006; Abdel *et al*., 2005).

Gonadosomatic Index; % GSI) of culturing male and female juvenile Tuptim tilapia

From (Appendix B. Table 26) and Figure 47, the GSI from male juvenile Tuptim tilapia in the cultures with 55%RS (0.94%), 50%RS (0.85%) and 45%RS (0.85%) were significantly higher than that in the culture with 0%RS at 150 days cultivation. The GSI from female juvenile Tuptim tilapia in the cultures with 55%RS (3.15%) and 50%RS (2.78%) were also significantly higher than those in the cultures with 45%RS and 0%RS at 150 days cultivation. The results from the present study were similar to the former study of spawning and egg quality of Nile tilapia fed solely on raw *S. platensis* throughout three generations where significant differences in fatty acid profile of the eggs were observed. The analyses of such study indicated that, within the group of fish fed by raw *S. platensis*, the eggs contained more linolenic acid, GLA, and $\sum$n-6 highly unsaturated fatty acids. It was also concluded that tilapia fed solely on raw *S. platensis* could keep normal reproduction throughout three generations (Lu and Takeuchi, 2004).

Analyses for physico-chemical water qualities in earthen ponds juvenile Tuptim tilapia

From (Appendix B. Table 27) and Figure 48, the water temperature and pH were ranged from 20.50 to 28.67 ^{o}C and from 6.93 to 7.30 units, respectively. The results from the present study were similar to the former study where most tilapia can adjust to a pH somewhat outside of their optimal range of 6.5-7.5 (Brian *et al*., 1986). The alkalinity of the water in the cultures with 55%RS (106 mg mL^{-1}), 50%RS (106 mg mL^{-1} and 0%RS (107 mg mL^{-1}) were significantly higher than that in the culture with 45%RS at 150 days cultivation. The TDS of the water in the cultures with

50%RS (133 mg mL^{-1}) and 45%RS (133 mg mL^{-1}) were significantly higher than those in the cultures with 55%RS and 0%RS (control) at 150 days cultivation. The conductivity of the water in the culture with 50%RS (347 $\mu S\ cm^{-1}$) and 55%RS (340 $\mu S\ cm^{-1}$ were significantly higher than that in the culture with 0% RS and 45%RS at 150 days cultivation. The DO of the water in the cultures with 55%RS (11.40 mg L^{-1}), 50%RS (11.20 mg L^{-1}) and 0%RS (10.90 mg L^{-1}) were significantly higher than that in the culture with 45%RS at 150 days cultivation. The results from the present study were similar to the former study which stated that surface waters designated as cold water fisheries must meet a minimum DO standard of 7 mg L^{-1}, while surface waters protected for warm water fish and aquatic life must meet a minimum DO standard of 5 mg L^{-1} (Clair *et al*, 2003).

The PO_4-P of the water in the culture with 0%RS (0.17 mg L^{-1}) was significantly higher than those in the cultures with 45%RS, 55%RS and 50%RS at 150 days cultivation. The NH_3-N of the water in the cultures with 0% RS (0.14 mg L^{-1}) and 55%RS (0.15 mg L^{-1}) were significantly higher than those in the cultures with 50%RS and 45%RS at 150 days. The results from the present study were similar to the former study which indicated that the range of PO_4-P in Indiana waters was quite broad range (0.01-0.17 mg L^{-1}) with a state average of 0.09 mg L^{-1}. The 0.03 mg L^{-1} is generally thought to indicate eutrophication potential. The Ohio Environmental Protection Agency found that the PO_4-P in streams that support modified warm water for fish was 0.28 mg L^{-1} (Stumm *et al*, 1996). According to the Indiana Administrative Code, maximum (unionized) NH_3-N concentrations should range between 0 to 0.21 mg L^{-1}. The present study was applied with the same evaluation of the effect upon temperature and pH (Wang *et al*., 1993).