CHAPTER 2

LITERATURE REVIEWS

2.1 Cultivation of *S. platensis*: nutritional value and water quality improvement

S. platensis is an attractive source of chlorophyll, a green pigment used in food, pharmaceutical and cosmetic industries, and other high-value cell components. *S. platensis* is also a rich source of protein for human consumption and animal feed purposes. This organism has a protein content that ranges from 60 to 72% (dry weight), 18% C-phycocyanin, 1.7% β-carotene, 1.6% chlorophyll- A and 26-30% γ- linoleic acid (GLA) as a percent of total fatty acid (Nakamura, 1982). *S. platensis* is an excellent source of C-phycocyanin. The protein fraction may contain up to 20% of C-phycocyanin. The C-phycocyanin has been used mainly as a food pigment, however, small quantities are included as biochemical tracers in immunoassays due to their fluorescent properties (Vonshak, 1997). Recently, it has been observed that C-phycocyanin has also shown anti-inflammatory and anti-cancer properties (Reddy *et al*., 2003). In this context, it has been described the antioxidant properties of a natural extract obtained from the blue–green alga in protecting human erythrocytes and plasma samples against oxidative damage *in vitro* (Lissi *et al*., 2000). The optimum conditions for extraction of C-phycocyanin from *S. platensis* were the highest biomass-solvent ratio of 0.08 g mL^{-1} and temperature 25 ^{o}C. Under these conditions it was possible to obtain an extract of C-phycocyanin with a concentration of 3.68 mg mL^{-1} and purity ratio (A615 / A280) of 0.46 (Silveira *et al*., 2007). The carotenoids that have been most studied in this regard are β-carotene, lycopene, lutein and zeaxanthin. In part, the beneficial effects of carotenoids are thought to be due to their role as antioxidants. β-carotene may have added benefits due to its ability to be converted to vitamin-A. These carotenoids are commercially available in supplement forms. However, intervention trials with large doses of β-carotene found an adverse effect on the incidence of lung cancer in smokers and workers exposed to asbestos (Norman and Elizabeth, 2005). β-carotene not only has antioxidant activity, at least *in vitro*, but it also enhances intercellular communication and may have immunomodulatory and anticarcinogenic activities in certain circumstances. However, the evidence for a unique role in human nutrition beyond its provitamin A function is, to date, not compelling (Hughes *et al*., 1997; Lee *et al*., 1999; Goodman, 2000). The freeze-dried biomass of *S. platensis* was submitted to supercritical CO_2 extraction for lipid and GLA yields. Using several conditions of ethanol 4.0% lipid content and 72.0% GLA were extracted (Bruno *et al*., 1993).

In Thailand, commercial *S. platensis* culturers use chemical nutrients. Raceway pond is more preferable than earthen pond for growing the algae because raceway pond can be cleaned and hence give better quality product eventhough more expensive. Total fatty acid content of *S. platensis* cultured in chemical composition in raceway pond has value of about 28.13% (Eugenia *et al*., 2001). *S. platensis* was autotrophically cultivated by fed-batch with urea added as a nitrogen source in raceway pond. Continuous and pulse feeding regimes of this nitrogen source (time intervals of 24 h) were compared using a constant mass flow rate. The intermittent addition of urea yielded results similar to those obtained by the continuous feeding, therefore, the former operation mode would be preferable to reduce the production costs of this cyanobacterium in large-scale facilities (Sanchez *et al*., 2004).

Interesting research work was carried out using animal wastes, wastewater and urban effluents as low-cost nitrogen sources. In batch and fed-batch cultures, large *S. platensis* biomass production was demonstrated along with elevated pigment content (Stanca and Popovici, 1996; Olguyn *et al*., 2000; Rangel-Yagui *et al*., 2004). Culturing *S. platensis* for animal feed purposes using inorganic culture media for the need to provide a full complement of nutrients has been relatively expensive. Low-cost alternatives, such as wastewater, should be evaluated as a more cost-effective method of producing this important nutritional product. Remediation of wastewater to remove unwanted nutrients using microorganisms such as algae has been widely applied throughout the world (Schlfsser, 1982). Production of *S. platensis* can therefore improve water quality, as demonstrated in a recent study in Mexico involving 50% swine waste in a suspended system. Significant algal biomass levels were obtained and wastewater nutrients levels were dramatically reduced (Canizares, 1993). In another work, maximum 96% total nitrogen (TN) and 54% total phosphorus (TP) were removed by *S. platensis* cultured in an outdoor raceway pond treating with 2% diluted anaerobic effluents from pig wastewater containing almost the same amount of nitrogen and phosphorus as in the experiment reported above (Matinez *et al*., 2000). These studies, along with the work using kitchen wastewater (Promya *et al*., 2006a), clearly demonstrate that *S. platensis* cultivation can be considered as a promising approach for nitrogen and phosphorus removal from wastewater (Olguyn *et al*., 2000; Chuntapa *et al*., 2003).

In China, wastewater arising from the production of sago starch had a high carbon/nitrogen ratio. This wastewater effluent with an average carbon C : N : P ratio of 24 : 6.14 : 1 could support the production of large amounts of *S. platensis* in ponds with an average specific growth rate of 0.51 g L^{-1} day^{-1},which 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 improvement of effluents (lower N, P) from a pig manure biogas digester by *S. platensis* was described in 1999 (Promya, 2000; Promya and Traichaiyaporn, 2003). The *S. platensis* biomass produced during the experiment could be used later as a dietary protein supplement in aquatic farming. All experimental ponds were continuously aerated. Water and algal samples were collected every 3 days over a culture period of 30 days. Over 98.99% of the ammonia nitrogen (NH_3-N) was removed in all treatments and dilutions of different effluents, thus meeting the pollution control standards in Thailand. The chemical oxygen demand (COD) was reduced by 97.13% in the 30% effluent dilutions, where final concentrations also met the standards. The highest primary biomass production (0.32 g L^{-1} as dry-weight) was achieved in the 50% effluent, where the chlorophyll- A content was between 38.6 and 69.2 μg L^{-1} (Promya and Traichaiporn, 2003).

S. platensis was co-cultured with black tiger shrimp (*Penaeus monodon*) for water quality control. The effects of: (1) three *S. platensis* trial conditions on inorganic nitrogen concentrations at one shrimp density (*S. platensis* trial conditions included: absent, nonharvested and semicontinuous harvesting) and (2) two shrimp densities on inorganic nitrogen concentrations, with and without *S. platensis*, were evaluated. Semi-continuous harvesting of *S. platensis* at one shrimp density resulted in significantly reduced ($p<0.05$) inorganic nitrogen concentrations (NH_3-N, nitrite nitrogen: NO_2-N and nitrate-nitrogen: NO_3-N). With *S. platensis* absence, NH_3-N and NO_2-N concentrations ranged from 0.5 to 0.6 mg L^{-1}, while NO_3-N concentration ranged from 16 to 18 mg L^{-1} by day 44. With non-harvested *S. platensis*, considerable variability occurred with nitrogen concentrations. Semicontinous harvest of *S. platensis* reduced NO_3-N to 4 mg L^{-1}, while NH_3-N and NO_2-N ranged from 0.0 to 0.15 mg L^{-1}, respectively (Chuntapa *et al*., 2003).

Improvement of the dormitory effluent from Maejo University, Chiang Mai, Thailand by cultivation of *S. platensis*, in 40, 70 and 100% of effluent, was studied (Promya and Traichaiyaporn, 2005a). This experimental work concerned the efficiency of *S. platensis* in removing nutrients (N, P) from the effluent leading to the reduction of water pollutants. All experimental batches were continuously aerated. Water and algal samples were collected every 3 days over a culture period of 20 days. By the end of the cultivation, *S. platensis* could remove the maximum of biochemical oxygen demand (BOD) from 40% wastewater. At 70% dilution, the maximum of COD, orthophosphate-phosphorus (PO_4-P) and NO_3-N were removed, whereas at the 100% dilution *S. platensis* could remove the maximum of NH_3-N. Statistical analysis confirmed that mean NH_3-N and PO_4-P in 40, 70 and 100% of the wastewater were lower than those in every control batch ($p<0.05$). Biomass and chlorophyll-A content in 100% wastewater were significantly higher than those cultivated in 40 and 70% wastewater ($p<0.05$). In conclusion, the 70% wastewater obtained from dormitory was the best concentration for *S. platensis* culture in order to improve water quality. However, the highest production was harvested from 100% wastewater.

The mass culture of *S. platensis* in kitchen wastewater (Kw, 90% and 100%) and oil-extracted soybean fermented water (Sw, 5% and 10%) were studied compared with those in a chemically defined medium modified Zarrouk's medium (modified Zm) (Promya *et al*., 2006). Biomass production and water quality from cultures of *S. platensis* were monitored every 5 day for 30 days. The highest primary production of *S. platensis* was achieved in 100%Kw and 5%Sw effluent (0.83 g L^{-1}). All experimental units had decreased NH_3-N, NO_3-N and NO_2-N contents to the levels where they had met the laws and standards of pollution control in Thailand (Promya *et al*., 2006a).

Factors affecting *S. platensis* growth and its chemical composition were undertaken. *S. platensis* was grown in open raceway ponds and dissolved oxygen (DO) concentration in the cultivation ponds was measured ranging between 10 mgL^{-1} in winter (115% of O_2 saturation) and 30 mg L^{-1} in summer (375% of O_2 saturation) and a clear decrease in biomass concentration was found when dissolved oxygen was >25 mg L^{-1} (Jime-neza *et al*., 2003). The culture medium of *S. platensis* using potassium nitrate with ammonium sulphate and urea as cheaper nitrogen sources has been investigated to give a high primary production (Solettoa *et al*., 2005). Evaluation of the effect of low light flux and nitrogen deficiency on growth and chemical composition of *S. platensis* in batch cultures utilizing a complex medium containing sea-water supplemented with anaerobic effluents from digested pig waste, was undertaken. Cultivation was carried out either at a light flux of 66 (lower) or 144 μmol photon m^{-2} s^{-1} (higher), utilizing bench raceways. Biomass concentration (as dry weight) after 12 days of cultivation in the complex medium was similar ($p<0.05$) to the one observed in Zarrouk's medium (Zm) regardless of the light intensity. Protein content of the biomass in the complex medium was significantly lower ($p<0.05$), compared to the Zm, regardless of the light flux. However, biomass from the complex medium was high in total lipids (28.6%) when cultures were exposed to the lower light flux. On the other hand, the palmitoleic acid percentage of total fatty acids was significantly higher ($p<0.05$) in a higher light intensity and a high level of GLA as a percentage of total fatty acids was observed (28.13%) in the biomass harvested from the complex medium in the lower light intensity. Finally, polysaccharide content was significantly higher ($p<0.05$) in the high light intensity and a very high content of total polysaccharides (28.41%) was observed in the complex medium (Eugenia *et al*., 2001).

2.2 Tubtim tilapia culture

In 2004 FAO reported Nile and Tuptim tilapias are popularly consumed and cultured extensively worldwide in Asia and Africa, especially in the tropical area and subtropical area. They are raised to substitute for other white flesh fish. Tilapias are the ninth ranking aquatic animals produced worldwide, more than Salmon, sea shrimp and mussel. The top nine aquatic animals ranking, from the highest to the lowest are: Pacific cupped oyster, Japanese carpet shell, Yesso scallop, Silver carp, Grass carp, Common carp, Bighead carp, Crucian carp and tilapia. Tilapia production worldwide in 1990 was just 830,000 tons. In 1999 the quantity increased up to 1.6 million tons; those captured from natural reservoirs were only 580,000 tons. Approximately 64% of tilapia production is derived from aquaculture. Asia is the largest producer providing 80% of total fish production. China is the top tilapia producer (35%) followed by Indonesia, Thailand, Philippines and Taiwan. The production from Africa and South America increases continually. Currently, culture of tilapia has continued to expand worldwide. Because the fish market in The U.S.A., Japanese and

European market expands continually, especially for the frozen tilapia fillet and other fish processing.

The U.S.A. is the top importer and consumer of tilapia. In The US, tilapia is the “fish of the 90’s.” In 1993, American annual per capita tilapia consumption was just 0.08 kg / person; however, Americans ate 0.19 kg / person in 1998. Since 1999, American tilapia consumption increases about 20% / year. For sea animals and trout, there is almost no increase in consumption. The ten tilapia exporters to the US include China, Taiwan, Ecudor, Costa Rica, Hondurus, Indonesia, Thailand, Panama, El Salvador. China is the major exporter, exporting about 90% of all products. The main production is that of whole frozen fish and the highest quality fish is exported to Japan in the form of fillet for sashimi (FAO, 2004).

The freshwater-reared red tilapia is a high-value species distributed mostly in urban specialty markets in the Philippines. Despite the high production cost in intensive culture, red tilapia farming is an economically feasible enterprise given the species’ domestic market price (at U.S $ 6.15/ kg; U.S $ 1.00 = Philippines peso 26.37) which is roughly twice as much as the Nile tilapia. Some red tilapia strains grow better in saline waters. Certain salt-tolerant strains like the Taiwanese red tilapia and the Florida red tilapia were found to be best suited for brackish/seawater culture (Watanabe *et al*., 1988a). In the U.S. and in the Caribbean Islands, considerable research has been done on the commercial culture of the Florida strain in fresh, brackish and sea water, in tanks, ponds and sea cages. Genetic strain variation in growth and other economically important traits like fecundity, feed conversion efficiency, red color inheritance (Avtalion and Reich, 1989; Koren *et al*., 1994) reproduction in high salinities, etc., have been demonstrated in earlier studies on the red tilapia. In comparisons of Taiwanese red tilapia an *Oreochromis mossambicus* X *O. niloticus* hybrid, Singapore red tilapia *O. mossambicus*, Philippines red tilapia an *O. mossambicus* X *O. niloticus* hybrid and F1 hybrids of *O. niloticus* and *O. aureus*, Pruginin *et al* (1988) showed that the Taiwanese red tilapia had the lowest growth and fecundity values while the Philippine red tilapia gave the highest response. On the other hand, when Taiwanese and Florida red tilapia were compared for breeding behavior in high salinities and only the Florida red tilapia was capable of reproducing in full-strength seawater (Watanabe *et al*., 1989a, b).

In the Philippines, several Asian red tilapia strains are available for culture. Earlier studies on some of these strains were on: (a) growth in lake-based net cages in the Philippines (Galman *et al*., 1988) and in brackish water ponds in Israel (Pruginin *et al*., 1988); (b) use of the red tilapia as a reference fish in Nile tilapia strain evaluation trials (Eguia and Eguia, 1993) and (c) breeding performance in different hatchery systems (Galman *et al*., 1988; Eguia, 1996). Tubtim tilapia has been considered promising for aquaculture because of its rapid growth, late reproduction and high multiplication rate. It has a firm consistences and tasty flesh of great market acceptance (Kubitza, 2000). It has been reported that some juvenile and adult fishes, including Tuptim tilapia, Nile tilapia and Java tilapia, are phytoplankton filters in the aquaculture system (Hakan *et al*., 2003). Most studies of phytoplankton in tilapia have been concentrated on descriptions of dietary preferences. There have been some quantitative studies on filter feeding of phytoplankton in tilapia, but most of them have mainly focused upon adult Nile tilapia. However, few data are available concerning the effect of phytoplankton on larval Nile tilapia. Much remains to be determined about how ingestion and assimilation of phytoplankton are affected by development of larval Nile tilapia and by different algal species (McDonald, 1987; Northcott *et al*., 1991).

Tuptim tilapia is known to change its diet and feeding mode from carnivorous to omnivorous at a standard length (SL) of 2–3 cm, and changes again to being a phytoplanktivorous filter feeder at about 6–7 cm SL in its natural habitat (Yada, 1982; Getachew, 1987 and McDonald, 1987). Studies have been ingestion and assimilation using three species of freshwater algae fed to larval Nile tilapia. Freshwater algae (*Spirulina platensis*, *Euglena gracilis* and *Chlorella vulgaris*) labeled with ^{14}C were fed to larva Nile tilapia (*Oreochromis niloticus*) with a standard length (SL) from 0.8 to 3.4 cm. This was conducted to clarify the acceptability of the three species of algae to developing larval tilapia based on their ingestion and assimilation of the ^{14}C-labeled algae. Ingestion rate (IR, Ag C/fish/h) and assimilation rate (AR, Ag C/fish/h), as well as ingestion efficiency (IE, %) and assimilation efficiency (AE, %) were investigated by monitoring the fate of the labeled food. Larval tilapia could ingestion rate 2.6–85% of the available *S. platensis* biomass with larvae growing from an SL of 0.8–3.4 cm. The relationship between IR and body weight (BW, mg) was expressed as IR = $15.62BW^{0.686}$ (r^2 = 0.94). Larva tilapia ingested significantly less *E. gracilis* (1.6–26% of the available *Euglena* biomass) than *S. platensis*, and the relationship between IR and BW was IR = $13.70BW^{0.584}$ (r^2 = 0.93). In contrast, a negligible amount (1.6–20% of the available *C. vulgaris* biomass) of *Chlorella* was ingested by larval tilapia over the range of sizes studied. For all three algae, a relatively lower assimilation efficiency (AE) was found in larvae right after the onset of exogenous feeding, but AE improved rapidly with increasing larva size from 0.8 to 1.2 cm SL. *S. platensis* was more readily assimilated (61.4–80%) than *Euglena* and *Chlorella*. Assimilated carbon from *Euglena* and *S. platensis* were about 1–2 and 3–6 times that of the resting carbon consumption requirement for respiration of the larval tilapia (Lu *et al*., 2004).

Phytoplankton is considered as a food supply for future manned space activities. A Closed Ecological Recirculating Aquaculture System (CERAS) is being studied to produce cost-efficient Nile tilapia with limited space, energy and labor by feeding them with phytoplankton (Takeuchi *et al*., 1997). Filtration of green algae and cyanobacteria by Nile tilapia, in the Partitioned Aquaculture System was studied. Nile tilapia held in a timed pulse fed Continuous Stirred Tank Reactor (CSTR) were provided Partitioned Aquaculture System (PAS) algal-rich water dominated by green algae (i.e., *Scenedesmus* and *Ankistrodesmus*) and cyanobacteria (i.e., *Microcystis* and *Merismopedia*) to determine filtration rates (FR). A similar number and size of tilapia were stocked at 1.5 kg/ tank into each of the six CSTRs (127 L) for 58-h experiment period. The cell counts of phytoplankton in water filtered by tilapia indicated significant reduction in green algae and cyanobacteria. Nile tilapia was more effective filtering the larger particle size taxa in both water sources. FR measured as mg of particulate organic carbon (POC) per kg fish wet weight per hour increased as POC increased. A curvilinear filter-feeding rate model provided a maximum filtration rate (FR_{max}) of 641 mgC kg^{-1} h^{-1} at 26 mgC L^{-1} in green algal-dominated water. The projected FR_{max} of cyanobacterial-dominated water was 865 mgC kg^{-1} h^{-1} at 59 mgC L^{-1}. The derived filter-feeding rate models will help to describe Nile tilapia filtration kinetics in the PAS and the potential for control of nuisance cyanobacteria (Hakan *et al*., 2003).

The study of spawning and egg quality of Nile tilapia fed solely on raw *S. platensis* throughout three generations revealed significant differences in the fatty acid profile of the eggs. In the group fed raw *S. platensis,* the survival rate of the larval fish was high and the eggs contained more GLA. It was also concluded that tilapia fed solely on raw *S. platensis* could maintain normal reproduction throughout three generations (Lu and Takeuchi, 2003). The effects of *S. platensis* supplement (0–20%) have also been assessed for chick's growth, egg production, egg quality, fertility, hatchability, and growth of the F1 generation. There were no significant differences due to the *S. platensis* content in any of the above parameters, except that the intensity of the yolk color increased with each succeeding level of *S. platensis* and fertility was higher for all *S. platensis* treatments versus the control (Ross and Dominy, 1990). The β-carotene from *S. platensis* for optimal growth of juvenile Nile tilapia was 28.6 to 44.3 mg kg^{-1} at a dietary vitamin-A content of 84 IU kg^{-1}. Tilapia is able to utilize β-carotene to fulfill the dietary vitamin-A requirements. The conversion ratio by weights of β-carotene to vitamin-A was approximately 19 : 1. The content of β-carotene in female juveniles Tuptim tilapia (42 mg 100 g^{-1}) was greater than that in male juvenile Tubtim tilapia (26 mg 100 g^{-1}) (Chien *et al*., 2006). The average water quality of tilapia culture in US of water temperature was between 28-32°C, pH: 6.0-8.5, dissolved Oxygen (DO): above 3.0 mg L^{-1}, alkalinity: 50-120 mg L^{-1}, NH_3-N: < 0.2 mg L^{-1}, PO_4-P: 0.1-0.5 mg L^{-1} (Tave, 1990).

Tuptim and Nile tilapias are the highest economic freshwater and the most produced species in Thailand. The production from culturing of tilapias in 2000 was 73,427.9 tons with 1,900.9 million baht in value. Thailand exports only 5% of tilapias production. For this reason, Thailand still has a chance to expand tilapia export because the tilapias market worldwide is continually growing (Nakano *et al*., 2003). The cost of nursing and culturing Tuptim tilapia in Thailand has been increases continually and the survival rate of this fish is normally low. Using a low cost and readily available natural feed to replace the commercial feed for Tuptim tilapia would lower the production cost. The effect of some raw *S. platensis* feeding on growth and blood count in fingerlings red tilapia (*Oreochromis* sp.) have been studied. The experiment was conducted in earthen ponds (5 x 5 m^2). Tilapia initially stocked were 0.5- 0.8 cm in length, 0.02-0.03 gm in weight and 500 fishes / m^2. Four treatments with three replications of each complete randomized design (CRD) were applied including T_1 (5% commercial diet / body weight / day), T_2 (10% raw *S. platensis* (0.1 $OD_{560\ nm}$) / body weight / day), T_3 (30% raw *S. platensis* (0.3 $OD_{560\ nm}$) / body weight / day) and T_4 (50% raw *S. platensis* (0.5 $OD_{560\ nm}$) / body weight / day). Data was collected every 15 - day for 90 days. The result showed that the specific growth rate and average daily growth of tilapia in T_4 and T_1 was significantly higher than the ones in T_2 and T_3 ($p<0.05$), respectively, but the survival rate of fish in T_4 was significantly higher than fish in T_3, T_2 and T_1 ($p<0.05$), in that order. Feed conversion rate, blood count after 60 days and 90 days of fish in T_3 and T_4 had significantly higher than fish in T_1 and T_2 ($p<0.05$) respectively. The cost of fingerlings / body and marginal rate of net return of fish in T_3 was better than for fish in $T_2 > T_1 > T_4$ ($p<0.05$), respectively. DO and NH_3-N in T_1 had significantly higher than T_2, T_3 and T_4 ($p<0.05$), respectively. PO_4-P with T_3 and T_2 had significantly higher than T_1 and T_4 ($p<0.05$), respectively (Promya *et al*., 2006a).

A 5–month feeding trail was carried out for red tilapia (*Oreochromis* sp.) with an initial average weight of 27 g for size 5 x 5 x 1 m in earthen ponds. Feeds containing varying percentages of raw *Spirulina platensis* 0, 45, 50 and 55 % were tested with three replications for each treatment. All the feeds were formulated to contain dietary requirement for the tilapia of 30% protein. The results showed that the feed with 55% raw *S. platensis* achieved the best performance survival rate and protein efficiency ratio. The nutritional value and total carotenoid contents in fish increased with the level of raw *S. platensis* in feed (Promya *et al*., 2006c). The study of improvement of meat quality of red tilapia has shown that carotenoid in the meat of red tilapia fed on commercial diets with 10% *S. platensis* was significantly higher than those fed on commercial diets with 5% *Cladophora* , 5% *S. platensis* and diets without algae (Promya *et al*., 2004).

Flesh quality improvement of Sharp Tooth African Catfish (*Clarias gariepinus*) using *Spirulina platensis* and *Cladophora* sp. has also been studied. Lead *S. platensis* and *Cladophora* sp. four experimental diets were used to fed: diets + 0% algae (T_1), diets+5% *S. platensis* (T_2), diets+10% *S. platensis* (T_3) and diets+5% *Cladophora* (T_4), respectively. The mass culture of Sharp Tooth African Catfish were fed in 30 m^2 fish ponds containing 300 fishes for 60 days. Results showed that the carotenoid contant in Sharp Tooth African Catfishs fed diets 5% *Cladophora* was significantly higher than those fed with diets with *S. platensis* 10%, *S. platensis* 5% and control diets [0% algae] ($p \leq 0.05$), respectively (Promya and Traichaiyaporn, 2005). The average water quality of tilapia culture in Thailand was found to have: water temperature between 28 to 32 °C, pH: 6.5 to 9, dissolved oxygen (DO): above 5.0 mg L^{-1}, biochemical oxygen demand (BOD): < 10.0 mg L^{-1}, alkalinity: 20 to 120 mg L^{-1}, NH_3-N: < 0.025 mg L^{-1}, PO_4-P: 0.1 to 0.5 mg L^{-1} (Tontulvet and Pornphrapa, 1982).

Nutritional values of tilapia had been assessed by various researchers with variation in some compositions. In 1995 Oonprasert reported the average nutritional values of the flesh of Nile tilapia were 17.29% protein, 0.80% fat, 1.37% of ash, 80.5% moisture and 0.09% carbohydrate. The crude protein contents of eggs from Tuptim tilapia were about 38 to 39%. The total ash of Tuptim tilapia egg had a value of about 12.9 to 14.4% dry weight (Abdel *et al*., 2005). The moisture of eggs Tuptim tilapia had a value of about 74.4 to 76.6 % wet weight and γ-linolenic acid for optimal growth of tilapia flesh was 3.5 to 5% (Chien *et al*., 2006).

One of the major factors influencing marketability of sea urchins is their gonad colour. The effects of a prepared diet, algal diets, and rotational feeding of these diet treatments on the European sea urchin *Paracentrotus lividus* were studied to determine a diet that would provide optimal gonad colour and gonadal somatic index (GSI = the gonad weight as a percent of the fish weight). *P. lividus* underwent six diet treatments: *Ulva lactuca* and *Gracilaria conferta* for 12 weeks (UG-12); prepared diet for 10 weeks followed by administration of *Ulva* and *Gracilaria* for 2 weeks (P-10); prepared diet for 8 weeks followed by *Ulva* and *Gracilaria* for 4 weeks (P-8); prepared diet for 6 weeks followed by Ulva and Gracilaria for 6 weeks (P-6); prepared diet for 12 weeks (P-12); and *Ulva*, *Gracilaria* and prepared diet for 12 weeks (UGP-12). Algae diet produced a dark orange colour but a low GSI. The pellet diet produced a good GSI but pale gonad colour. *P. lividus* fed the prepared diet for 8 weeks followed by 4 weeks of algal diet produced the optimal combination of desired gonad colour and GSI. The dominant type of carotenoid in the gonad is echinenone, which the sea urchin synthesises from β-carotene. The higher echinenone level in the gonad, the more intense its colouration. Low levels of echinenone were found in the gut with its high accumulation in the gonads, the reverse of the β-carotene profile, indicating bioconversion within the gonad or upon transfer from the gut to the gonad (Muki *et al*., 2005).

2.3 The immunity

Millions of people worldwide eat *S. platensis* cultivated in scientifically designed algae farms. Current world production of *S. platensis* for human consumption is more than one thousand metric tons annually. The U.S.A. leads world production followed by Thailand, India and China. More countries are planning production as they realize that it is a valuable strategic resource. *S. platensis* is a powerful tonic for the immune system. In scientific studies of mice, hamsters, chickens, turkeys, cats and fish, *S. platensis* consistently improves immune system function. Medical scientists find *S. platensis* not only stimulates the human immune system but it also actually enhances the body's ability to generate new blood cells (Babu, 1995). Important parts of the immune system (Figure 5), the bone marrow stem cells, macrophages, T-cells and natural killer cells, exhibit enhanced activity with *S. platensis*. The spleen and thymus glands also show enhanced function. Scientists have also observed that *S. platensis* causes macrophages to increase in number, become "activated" and more effective at killing germs (Babu, 1995). Feeding studies show that even small amounts of *S. platensis* build up both the humoral and cellular arms of the immune system. *S. platensis* accelerates production of the humoral system, (antibodies and cytokines), allowing it to better protect against invading germs. The cellular immune system includes T-cells, macrophages, B-cells and the anti-cancer natural killer cells. These cells circulate in the blood and are especially rich in body organs like liver, spleen, thymus, lymph nodes, adenoids, tonsils and bone marrow. *S. platensis* up-regulates these key cells and organs, improving their ability to function in spite of stresses from environmental toxins and infectious agents (Pang, 1988; Qureshi and Ali, 1995).

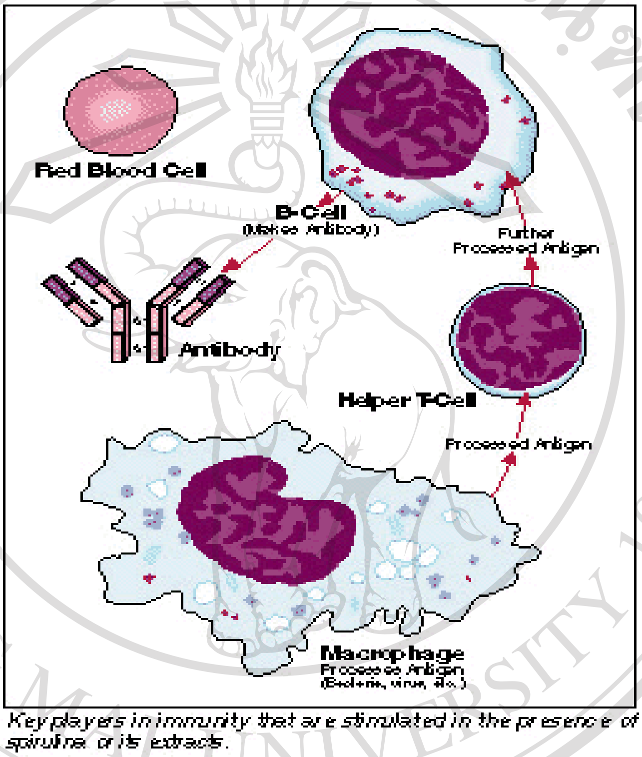

Figure 5 Important parts of the immune system (Babu, 1995)

S. platensis acts as a functional food, feeding beneficial intestinal flora, especially *Lactobacillus* and *Bifidus*. Maintaining a healthy population of these bacteria in the intestine reduces potential problems from opportunistic pathogens like *E. coli* and *Candida albicans*. Studies show that when *S. platensis* is added to the diet, beneficial intestinal flora increase (Ayehunie *et al*., 1996). Several studies show *S. platensis* or its extracts can prevent or inhibit cancers in humans and animals. Some common forms of cancer are thought to be a result of damaged cell DNA running amok, causing uncontrolled cell growth. Cellular biologists have defined a system of special enzymes called endonucleases which repair damaged DNA to keep cells alive and healthy. When these enzymes are deactivated by radiation or toxins, errors in DNA go unrepaired and cancer may develop. *In vitro* studies suggest the unique polysaccharides of *S. platensis* enhance cell nucleus enzyme activity and DNA repair synthesis. This may be why several scientific studies observing human tobacco users and experimental cancers in animals report high levels of suppression of several important types of cancer. The subjects were fed either whole *S. platensis* or treated with its water extracts (Pang, 1988; Lisheng, 1991).

The evaluation of blood chemistry parameters in animals is a routine and important tool in clinical veterinary medical practice. This simple technique can provide essential information on the physiological status of animals and therefore help the clinician to make proper medical decisions. In fish, however, the predictive value is compromised by the lack of reliable normal databases and available reference laboratories to properly analyze these samples (Groff and Zinkl, 1999). Moreover, the relationship between certain diseases or pathological changes and changes in blood chemistry parameters has not been fully established. Those blood chemistries for which a relationship with a disease state has been established for major cultured fish species (Casillas *et al*., 1983).

The effects of commercially available green algae extract *Dunaliella* on the growth, immune functions and disease resistance were determined in black tiger shrimp (*Penaeus monodon*). Trial I was performed on small shrimp (1–2 g body weight). Shrimp fed with 125–300 mg of the *Dunaliella* extract per one kg diet for 8 weeks showed higher weight gain and survival compared to the control. There was no significant difference in total hemocyte count and phenoloxidase activity in this treatment. Shrimp fed with 300 mg of the extract per one kg diet exhibited higher resistance to WSSV (white spot syndrome virus) infection than other groups and also became more tolerant of the low dissolved oxygen stress. Color intensity of boiled shrimp was highest in groups fed with 200–300 mg of the *Dunaliella* extract/kg diet. Trial II was performed in juvenile shrimp (12–15 g body weight) fed with the same diet as in Trial I for 6 weeks. Total hemocyte counts were negatively correlated to the increase in *Dunaliella* extract in test diet (r = - 0.97). Phenoloxidase activity and clearance of pathogenic vibrio from the hemolymph were not significantly different. Total carotenoid and astaxanthin levels were highest in shrimp fed with 200–300 mg of the *Dunaliella* extract. In conclusion, the *Dunaliella* extract showed beneficial effects as a shrimp feed supplement (Kidchakan *et al*., 2005).

Lysozyme is an enzyme which acts as a hydrolase by breaking down the beta-(1-4) linkages in the peptidoglycan layer of bacterial cell walls. Bacteria are either destroyed directly or are opsonize, enabling its destruction through phagocytosis. While gram positive bacteria are more sensitive to lysozyme than gram negative bacteria in mammals, fish lysozyme is capable of responding to both gram positive and gram negative bacteria. Lysozyme often works simultaneously with other mechanisms of the non-specific immune system, such as complement and macrophage activity. Lysozyme is continuously expressed by neutrophils, monocytes and macrophages, although lysozyme activity has been found to increase with a stimulation of the immune system. Changes in lysozyme activity are common once a fish becomes infected with a pathogen, depending on the level of infection. Lysozyme in fish is commonly found in the kidney, spleen, skin mucus, gills, blood plasma, and ovaries. The enzyme may act as a first encounter defense to bacteria by existing externally on the fish, such as in the skin mucus and gills. These defense strategies, along with the internal function of lysozyme within the major organs, contribute greatly to preventing bacteria from causing infection before it starts (Hayashi and Hayashi., 1996).

Lysozyme in fish can be measured either by the turbidimetric method or the agarose plate assay. Each method was developed based on the amount of lysis of the gram positive bacteria *Micrococcus lysodeikticus*. The turbidimetric assay measures lysozyme activity according to the decrease in light absorbance (increase in optical density). Lysozyme is measured according to the area cleared by lysis in the lysoplate assay. There is an inverse relationship between lysozyme activity and the amount of existing bacteria in both assays. In addition, a known amount of hen egg white lysozyme is used as a standard for both assays (Sardar *et al*., 2001).