International Journal of Agricultural Technology 2013 Vol. 9(1): 49-59
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Enhancement of carotenoid production in *Spirulina platensis* and fed on *Clarias macrocephalus* for reproductive performance

Thaweedet Chainapong and Siripen Traichaiyaporn*

Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

Thaweedet Chainapong and Siripen Traichaiyaporn (2013) Enhancement of carotenoid production in *Spirulina platensis* and fed on *Clarias macrocephalus* for reproductive performance. International Journal of Agricultural Technology 9(1):49-59.

Abstract The mixotrophic culture of *Spirulina platensis* supplemented with acetate as organic carbon source (1.0-4.0 g L^{-1} acetate) to improve the production of algal biomass and carotenoids was investigated. Results indicated that the higher acetate concentration increased biomass and specific growth rate, whereas lower acetate concentration gave high amount of carotenoids. It was also found that *S. platensis* was a source of provitamin A. The effect of dietary *S. platensis* on carotenoids content in flesh, ovary and reproductive performance of female walking catfish (*Clarias macrocephalus*) were investigated. Three experimental diets were tested to contain *S. platensis* at 0% (control), 5% and 10%. The amount of flesh carotenoids in fish fed with diet containing *S. platensis* increased but no significant when compared with the control diet. However, the highest amount of carotenoids 3.0 mg $100g^{-1}$ dry weight of ovary in fish fed with supplemental 10% *S. platensis* was significantly higher (p<0.05) than in the other experiments. There was no significant difference in the reproductive performance and egg quality among fish fed with *S. platensis* supplement and control diet.

Key words: *Spirulina platensis*, Carotenoids, *Clarias macrocephalus*, Reproductive performance

Introduction

Spirulina platensis is generally cultured for algal health food because of its high-value of pigments, protein, fatty acids and other nutritional elements which can be used in food, pharmaceutical and cosmetic industries (Singh *et al*., 2005). The carotenoids from this alga contain high level of β–carotene and zeaxanthin (Lorenz *et al*., 2005). *S. platensis* has been found to consume inorganic and organic carbon sources for mixotrophic growth. The biomass produced during mixotrophic growth increase when compared with that in photoautotrophic growth (Singh *et al*., 2005; Vonshak *et al*., 2000). The

* **Corresponding author**: Siripen Traichaiyaporn; **e-mail:** tsiripen@yahoo.com

mixotrophic culture led to a significant enhancement in biomass concentration and production of photosynthetic pigments; lutein, β-carotene, phycocyanin and allophycocyanin (Chen *et al*., 2006). Carotenoids were reported to be vitamin A precursors in human and animals (Matsuno, 2001). When no vitamin A was supplemented, dietary β-carotene significantly influenced the growth of juvenile hybrid tilapia (Hu *et al*., 2006). *S. platensis* is used as a natural source of carotenoids in feed supplement for pigment accumulation in ornamental fish such as Japanese ornamental carp (Cyprinus carpio L.) (Sun *et al*., 2012). Furthermore, *S. platensis* affected the reproduction in hybrid Tuptim tilapia ND56 (*Oreochromis* sp.) (Promya, 2008). Supplement in pellet feed can also improve the growth and maturation performance the brood stock of Mekong giant catfish, *Pangasinodon gigas*, (Meng-umphan and Saengkrachang, 2008). The carotenoids are also associated with reproductive organs and enhance the hatching success and survival of alevins (Shahidi *et al*., 1998).

In Thailand, the flesh of walking catfish (*Clarias macrocephalus*) is tender and has a better taste then a high-valve species which is popular in earthen pond culture. However, the hybrid catfish, *C. macrocephalus* (♀) × *C. galipinus* (♂) is the most popular in aquaculture because it has a high growth rate and is more disease resistant (Petkam and Moodie, 2001). While, the population of walking catfish has decreased, it is important to improve the reproductive performance of female catfish for future demand of the hybrids. The objectives of this study were to investigate the carotenoid content of *S. platensis* culture at different mixotrophic status and the optimal condition was selected for mass cultivation to use *S. platensis* as a diet supplement for the female walking catfish and to be treated the carotenoids content of the flesh and gonads as well as. Finally, determine changes in reproductive performance from the gonadosomatic index (GSI).

Materials and methods

S. platensis was obtained from Thailand Institute of Scientific and Technological Research (TISTR). The alga was cultured in 9 L glass tanks containing 5 L of Zarrouk's medium, consisting of (L^{-1}) 16.8 g $NaHCO_3$, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$, 1.0 g K_2SO_4, 1.0 g NaCl, 0.2 g $MgSO_4.7H_2O$, 0.04 g $CaCl_2.2H_2O$, 0.2 g $FeSO_4.7H_2O$, 1.6 g $Na_2EDTA.2H_2O$. The medium was supplemented with sodium acetate (CH_3COONa) at different concentrations (0-4 g L^{-1}) as carbon source. An initial pH was 9.0±0.5 and air pump was operated. Light-shade was adapted 50% of natural sun light. Each treatment had three replicates. The growth of *S. platensis* was determined by measuring the optical density at 680 nm using a HACH/DR 2500 spectrophotometer, linear

regression relating optical density to dry weight (g L^{-1}) (Olaizola and Duerr, 1990). The maximum *S. platensis* biomass concentration (X_{max}, g L^{-1}), biomass productivity (P_x, g $L^{-1}d^{-1}$) was calculated from the equation $P_x = (X_i-X_o)/t_i$, where X_o = initial biomass concentration (g L^{-1}), X_i = biomass concentration at time i (g L^{-1}) and t_i = time interval (day). Maximum specific growth rate (μ_{max}, d^{-1}) was calculated from the equation $\mu_{max} = (\ln X_2-\ln X_1)/t_2-t_1$, where X_2 and X_1 are biomass concentrations at time intervals t_2 and t_1 and doubling time was also calculated (Andrade and Costa, 2007; Trabelsi *et al.*, 2009). Carotenoids and individual carotenoids were separated from the freeze dried cells.

The optimal condition was used for mass culture of *S. platensis* in the 300 L concrete circular pond and harvested once a week by filtering with nylon mesh and hot air oven dried at 70°C. Fish diet containing with 0% (control), 5% and 10% S. *platensis* were prepared to contain 36% crude protein (Table 1). All experimental diets were air dried and stored in the dark at room temperature.

Table 1. Ingredient and proximate composition of the experimental diets (g $100g^{-1}$ diet) containing 0%, 5% and 10% *S. platensis*

Ingredient (g $100g^{-1}$)	**Dietary treatment**		
	0%	5%	10%
Rice	14.0	12.0	12.0
Soybean meal	30.0	31.0	32.0
Rice bran	21.0	22.0	21.0
Fish meal	32.0	27.0	22.0
Premix	1.0	1.0	1.0
Spirulina	0.0	5.0	10.0
Soybean oil	2.0	2.0	2.0
Proximate composition			
(% dry weight)	35.91±1.00	36.74±2.18	36.67±1.77
Crude protein	10.09±0.23	10.20±0.93	9.83±0.25
Crude fat	3.90±0.02	3.73±0.19	3.63±0.43
Crude fiber	10.62±0.04	10.57±0.12	10.02±0.08
Ash	7.54±0.09	7.65±0.32	7.86±0.26
Moisture	39.48±1.21	38.76±2.86	39.85±1.42
NFE *			

* Nitrogen-free extract = 100 – (protein + fat + ash + fiber).

Four-month-old female *C. macrocephalus* grown with a commercial diet in a local fish farm were purchased and used in these experiments. The average size of its fish was 13 cm and the body weight was 19 g. Three treatments with each three replicates were varied different diet of *S. platensis* in feed. This fish

was cultured in floating cages of nylon nets (6 m^3, 2×3×1 m) prepare of suspended inside an earthen pond. Fifty fishes were in each cage (8.3 fish per m^3) and fed with prepare at 5% of their body weight twice a day, between 8.00 – 9.00 a.m. and 5.00 – 6.00 p.m. for 4 months. Each month three fishes from each cage were sacrificed to determine the gonadosomatic index (GSI) = (gonad weight (g) × 100) / Fish weight (g).

Crude protein, lipid, fiber, ash and moisture contents of feed ingredients were determined by standard AOAC methods (AOAC, 1995). Total carotenoids from *S. platensis*, experimental diets, flesh and ovary of female walking catfish were determined by the spectrophotometric method (Britton, 2005). Individual carotenoids including β-carotene, zeaxanthin, lutein and astaxanthin were extracted and analyzed by high performance liquid chromatography (HPLC) using a YMC carotenoid column S5 (4.6 x 250 mm) (Inbaraj *et al*., 2006) and a mobile phase consisting of methanol:chloroform (80:20). The flow rate was 1.0 ml min^{-1}. These pigments were detected by a UV-Vis absorbance at 456 nm.

Analytical standards of β-carotene, zeaxanthin, lutein and astaxanthin were obtained from Sigma Chemical Company. Vitamin A activity, as retinal equivalents (RE) was calculated from the amount of β-carotene (1 RE = 6 μg β-carotene).

All the experiments were performed with three replicates. One-way ANOVA and Duncan's multiple range test (DMRT) were applied for used to determine the mean value, standard deviation and significant differences among the treatments at $p<0.05$.

Results and discussions

The growth of *S. platensis*; maximum biomass concentration (X_{max}), biomass productivity (P_x), maximum specific growth rate (μ_{max}) and doubling time (t_d) show not significant ($p>0.05$) difference among the treatments (Table 2). In mixotrophic culture with 4.0 g L^{-1} acetate gave the highest algal biomass (0.87 g L^{-1}), biomass productivity (0.05 g $L^{-1}d^{-1}$) and specific growth rate (0.13 d^{-1}) on day 13. While, mixotrophic culture with 3.0 g L^{-1} acetate and control gave lower biomass concentration was 0.72 g L^{-1}. Mixotrophic culture with acetate provided the *S. platensis* enough carbon source and energy to support the cell growth (Chen *et al*., 2006). Therefore, the cells grown under mixotrophic culture grew faster and high biomass concentration (Vonshak *et al*., 2000). Previous reported high biomass concentration than in present study. Chen *et al*. (2005) reported that the addition of glucose and acetate for mixotrophic culture enhance of biomass concentrations 2.57 and 1.65 g L^{-1}, respectively. During the study, light and temperature varied with diurnal

change, media temperature (27.0 – 31.0 ˚C), light intensity (3,000 – 541,000 Lux) and photoperiod about 12 h:12 h light:dark cycle (data not shown). Previous study was found that the effect of high light intensity was inhibitory to the growth of *Spirulina* (Chen *et al*., 1996 and Zhang *et al*., 1999). Furthermore, light intensity and glucose concentration combined affected the growth of *S. platensis* (Rym *et al*., 2010); found that low light intensity glucose had a low effect on biomass production and photosynthetic rate. At the highest light intensity glucose was more sensitive all responses.

Table 2. Maximum biomass concentration (X_{max}), biomass productivity (P_x), maximum specific growth rate (μ_{max}), doubling time (t_d) and pH for *S. platensis* cultivated under photoautotrophic and mixotrophic condition

Acetate (g L^{-1})	**X_{max} (g L^{-1})**	**P_x (g $L^{-1}d^{-1}$)**	**μ_{max} (d^{-1})**	**t_d (day)**	**pH**
0.0	0.72±0.02	0.04±0.00	0.12±0.00	6.02±0.38	9.56±0.04^{bc}
1.0	0.77±0.04	0.05±0.00	0.12±0.00	5.68±0.12	9.61±0.02^{c}
2.0	0.74±0.16	0.05±0.02	0.12±0.03	6.11±1.22	9.59±0.07^{c}
3.0	0.72±0.05	0.04±0.01	0.12±0.02	6.04±1.09	9.50±0.03^{ab}
4.0	0.87±0.04	0.05±0.00	0.13±0.00	5.43±0.07	9.46±0.05^{a}

Mean values ± S.D. in column with different superscripts were significant different (p<0.05).

The effect of acetate concentration on carotenoids production of *S. platensis* is shown in Table 3. The cells grown under mixotrophic condition did not produce significant amount of carotenoids compared with those in the photoautotrophic condition (control). The high amount of carotenoids was obtained when the alga was grown in mixotrophic condition with 1.0 g L^{-1} acetate (6.62 mg g^{-1}). The addition of high acetate concentration, particularly 4.0 g L^{-1} acetate was decrease of the amount of carotenoids (5.75 mg g^{-1}). It was observed that the production of individual carotenoid varied with the growth condition by supplementation of acetate. The growth of photoautotrophic culture produced lower amount of zeaxanthin and β-carotene were 0.36 and 0.33 mg g^{-1}, respectively. Increasing acetate concentration, 2.0 and 3.0 g L^{-1}, were significantly (p<0.05) increased the amount of zeaxanthin (1.69 and 1.78 mg g^{-1}, respectively) and β-carotene (0.69 and 0.68 mg g^{-1}, respectively). However, the highest acetate supplement 4.0 g L^{-1} was found to decrease zeaxanthin and β-carotene (1.26 and 0.59 mg g^{-1}, respectively). Lutein was unaffected with the addition of acetate. It was who found that *S. platensis* produced a small amount of lutein. The growth of *S. platensis* in mixotrophic condition with the addition of acetate significantly increased the amount of β-carotene when compared with that in photoautotrophic condition (Chen *et al*.,

2006). While, decrease in zeaxanthin and β-carotene with the addition of higher acetate concentration (4.0 g L^{-1}) was probably due to the inhibitory effect of by carbon source (Rym *et al*., 2010). In the present study highest vitamin A activity (retinol equivalent, RE) were 116 and 113 µg RE significantly (p<0.05) enhanced by 2.0 and 3.0 g L^{-1} acetate, respectively.

Table 3. Amount of carotenoids, lutein, zeaxanthin, β-carotene (mg g^{-1}) and vitamin A activity (µg RE) was obtained in the *S. platensis* cultivated under mixotrophic condition with acetate (g L^{-1})

Acetate	Carotenoids	Lutein	Zeaxanthin	β-carotene	vitamin A*
0.0	6.30±0.53	0.023±0.01	0.36±0.20^{a}	0.33±0.05^{a}	56±8^{a}
1.0	6.62±0.26	0.023±0.00	0.96±0.35^{b}	0.59±0.04^{b}	98±6^{b}
2.0	5.81±0.31	0.018±0.00	1.69±0.42^{cd}	0.69±0.05^{c}	116±9^{c}
3.0	6.15±0.26	0.017±0.00	1.78±0.08^{d}	0.68±0.05^{c}	113±8^{c}
4.0	5.75±0.51	0.021±0.00	1.26±0.04^{bc}	0.59±0.03^{b}	98±5^{b}

Mean values ± S.D. in column with different superscripts were significant different (p<0.05). * 1 RE = 6 µg β-carotene

The dietary of *S. platensis* on carotenoid content and reproductive performance in female walking catfish (*Clarias macrocephalus*) was investigated. The proximate compositions of experimental diets show in Table 1. Diets were prepared to be isonitrogenous and contained about 36% crude protein. The amount of carotenoids and individual carotenoid from experimental diets were extracted as show in Fig. 1. Comparison of various diets indicated that 10% *S. platensis* had the highest content of total carotenoids, lutein, zeaxanthin, β-carotene and vitamin A, while the control diet was found to have very low amount of carotenoids and only lutein was detected.

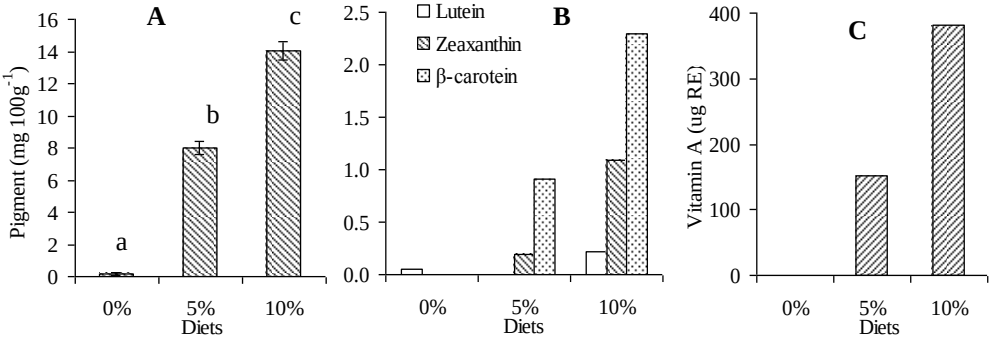

Fig. 1. Amount of carotenoids (A), lutein, zeaxanthin, β-carotene (B) and vitamin A (C) in three experimental diets supplemented with 0%, 5% and 10% *S. platensis*. Statistical differences between groups are indicated by different letters (p < 0.05).

The amount of carotenoids, lutein, zeaxanthin and β-carotene in the flesh and ovary of walking catfish are presented in Table 4. In initial experiment the amount of carotenoids in flesh of walking catfish was 0.54 mg $100g^{-1}$ dry weight (DW). After 4 months of feeding with *S. platensis* (0%, 5% and 10%) supplement in the diets, it was found that amount of carotenoids in the flesh was 0.96, 1.20 and 1.20 mg $100g^{-1}$ DW, respectively, which were higher than that in the initial experiment. However, the amount of carotenoids was not significantly (p>0.05) different among treatment. The fish with fed 10% *S. platensis* the fish ovary carotenoids was 3.01 mg $100g^{-1}$ DW which was significant (p<0.05) higher than those fish fed diets supplement with 5% *S. platensis* and the control group, 1.25 and 1.22 mg $100g^{-1}$ DW, respectively. Analysis of individual carotenoid the flesh revealed that the fish fed with supplemental 5% *S. platensis* accumulated lutein and zeaxanthin (0.08 and 0.25 mg $100g^{-1}$ DW, respectively) significantly (p<0.05) higher than in other treatments. Whereas, 10% *S. platensis* diet showed highest β-carotene content of 0.51 mg $100g^{-1}$ DW. In comparison with individual carotenoid of ovary in the fish fed with supplemental 10% *S. platensis* the amount of lutein (0.13 mg $100g^{-1}$ DW) was significant higher (p<0.05) than that in other group. While, the fish fed with 5% *S. platensis* the amount of zeaxanthin and β-carotene were 0.054 and 0.080 mg $100g^{-1}$ DW, respectively, which were significantly higher (p<0.05) than those in other experimental conditions.

Table 4. Effect of dietary treatment on the concentration of total carotenoids, lutein, zeaxanthin and β-carotene (mg $100g^{-1}$, mean±S.D. of three replication) in flesh and ovary of female walking catfish

Experimental	Diet	Carotenoids	Lutein	Zeaxanthin	β-carotene
Flesh	initial	0.54±0.13	ND	ND	ND
	0%	0.96±0.21	$0.054±0.00^{b}$	$0.048±0.00^{b}$	ND
	5%	1.20±0.24	$0.080±0.00^{c}$	$0.25±0.00^{c}$	ND
	10%	1.20±0.26	$0.024±0.02^{a}$	$0.032±0.00^{a}$	0.51±0.01
Ovary	0%	$1.22±0.13^{a}$	$0.097±0.00^{b}$	$0.051±0.00^{ab}$	$0.05±0.00^{a}$
	5%	$1.25±0.34^{a}$	$0.094±0.00^{a}$	$0.054±0.00^{b}$	$0.08±0.00^{b}$
	10%	$3.01±0.13^{b}$	$0.13±0.00^{c}$	$0.048±0.00^{a}$	ND

Mean values ± S.D. in column with different superscripts were significant different (p<0.05). ND = not determined

One month from the experiment the filamentous green alga *Sirogonium* sp. was found in the floating cages and the stomach contents of walking catfish also contained this alga and other micro algae e.g. *Surirella* sp. and *Nitzchla* sp. etc. This indicates that fish carotenoids in control diet obtain from algae, environmental source of pigments (Welker *et al*., 2001). However, carotenoid

accumulation in fish flesh and ovary also from fed *S. platensis* supplement in feed. Other research reported that carotenoid contents in the flesh of hybrid catfish increased with the level of *Spirulina* sp. supplemented (Phromkunthong and Pipattanawattanakul, 2005).

The reproductive performance of female walking catfish 4 months after feeding in three experimental groups is shown in Table 5. There was no significant (p>0.05) difference in fish and ovarian weight among treatments. Fish fed with 0% (control diet), 5% and 10% of *S. platensis* showed mean fish weight of 81.4, 77.6 and 77.7 g, respectively. The result showed that the highest fish weight after fed control diet than fed the diets supplement with *S. platensis*. However, in the fish fed with control diet the ovarian weight (7.95 g) was lower than fish fed that of with supplemental 10% and 5% *S. platensis* (9.25 and 10.24 g, respectively). All experimental diets showed no significant (p>0.05) difference in gonadosomatic index (GSI). There was relationship between the ovarian weight and GSI (r=0.933, p<0.01). The dietary treatments with supplement 0%, 5% and 10% *S. platensis* did not significantly affect (p>0.05) the number of egg g^{-1} fish weight and number of egg g^{-1} spawn. However, significant (p<0.05) difference was found the egg diameter. Fish fed with 10% *S. platensis* showed the longer egg diameter.

Table 5. Effect of different levels of dietary treatments on fish weight, ovarian weight, gonadosomatic index (GSI), Number of eggs g^{-1} fish weight, Number of eggs g^{-1} spawn and Egg diameter

Diet	Fish weight (g)	Ovarian weight (g)	GSI (%)	Number of eggs g^{-1} fish weight	Number of eggs g^{-1} spawn	Egg diameter (mm)
0%	81.4±16.1	7.95±0.52	11.14±3.89	56±8	444±59	0.66±0.03[a]
5%	77.6±13.9	10.24±3.44	10.14±4.02	57±18	460±31	0.66±0.02[a]
10%	77.7±13.7	9.25±1.43	11.01±3.79	52±12	438±68	0.68±0.02[b]

Mean values ± S.D. in column with different superscripts were significant different (p<0.05).

The present study showed that the reproductive performance slightly higher than reported by Na-Nakorn (1995) who observes a GSI 8.8% at 1 year of age in walking catfish. Whereas, similar results in the fecundity (no. of eggs g^{-1} fish weight and no. of eggs g^{-1} spawn) which reported by Ali (1993) and Santiago and Gonzal (1997). There are also reports that reproductive performance is related to the age of the fish and the breeding season. The addition of *S. platensis* to the diet has show an increase amount of caronoids (precursor of vitamin A) in fish flesh and ovary. The effect of vitamin A supplementation in broodstock feed on reproductive performance was reported

by Palace and Werner (2006). They showed that the dietary content of vitamin A transfer to oogenesis is an important determinant of reproductive performance. In the present study, it was found that no significant difference among the treatments for reproductive performance and egg quality of female walking catfish after fed *S. platensis* supplement diet. However, there is a possibility for use as a source of carotenoids for increased efficiency in the maturation.

Conclusion

Supplementation of acetate to the culture medium show no significant difference in the production of biomass and carotenoids concentration, but it affected the amount of zeaxanthin and β-carotene in *S. platensis*. It was also found that the addition 2.0 and 3.0 g L^{-1} acetate were increased the amount of zeaxanthin and β-carotene. The cultivation of female walking catfish using *S. platensis* as feed supplement to increase carotenoids in flesh and ovary. The amount of β-carotene, lutein and zeaxanthin were accumulated in flesh and ovary after fed with supplemental 5 and 10% *S. platensis* in the diets. However, it had no effect on the reproductive performance and egg quality. For future research on using *S. platensis* as carotenoid source for female brood walking catfish should be conducted by additional higher algal level.

Acknowledgements

This research was supported by the CHE Ph.D. Scholarship program Office of the Higher Education Commission, Bangkok, Thailand

References

Ali, A.B. (1993). Aspects of fecundity of the feral catfish, *Clarias macrocephalus* (Gunther), population obtained from the rice fields used for rice-fish farming, in Malaysia. Hydrobiologia 254:81-89.

Andrade, M.R. and Costa, J.A.V. (2007). Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. Aquaculture 264(1-4):130-134.

AOAC. (1995). Official Methods of Analysis of AOAC International. 16th edition. United States of America.

Britton, G. (2005). Workshop on Carotenoids: The Qualitative and Quantitative Analysis. Prince of Songkla University. Songkla.

Chen, F., Y., Zhang and Guo, S. (1996). Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. Biotechnology Letters 18(5):603-608.

Chen, T., W., Zheng, Y.-S., Wong, F., Yang and Bai, Y. (2005). Accumulation of selenium in mixotrophic culture of *Spirulina platensis* on glucose. Bioresource Technology 97:2260-2265.

Chen, T., W., Zheng, F., Yang, Y., Bai and Wong, Y.S. (2006). Mixotrophic culture of high selenium-enriched *Spirulina platensis* on acetate and the enhanced production of photosynthetic pigments. Enzyme and Microbial Technology 39:103-107.

Hu, C.J., S.M., Chen, C.H., Pan and Huang, C.H. (2006). Effect of dietary vitamin A or β-carotene concentrations on growth of juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture 253:602-607.

Inbaraj, B.S., Chien, J.T. and Chen, B.H. (2006). Improved high performance liquid chromatographic method for determination of carotenoids in the microalga *Chlorella pyrenoidosa*. Journal of Chromatography A, 1102:193-199.

Lorenz, M., T., Friedl and Day, J.G. (2005). Perpetual maintenance of actively metabolizing microalgal cultures. Algal Culturing Techniques. Elsevier Academic Press, Burlington, USA, pp. 145-156.

Matsuno, T. (1991). Xanthophylls as precursors of retinoids. Pure & Apply Chemical 63(1):81-88.

Meng-umphan, K. and Saengkrachang, J. (2008). Production of Generation-2 Mekong giant catfish (*Pangasinodon gigas*) cultured with *Spirulina* sp. Maejo International Journal of Science and Technology 2(3):559-567.

Na-Nakorn, U. (1995). Comparison of cold and heat shocks to induce diploid gynogenesis in Thai walking catfish (*Clarias macrocephalus*) and performances of gynogens. Aquatic Living Resources 8:333-341.

Olaizola, M. and Duerr, E.O. (1990). Effects of light intensity and quality on the growth rate and photosynthesis pigment content of *Spirulina platensis*. Journal of Applied Phycology 2:97-104.

Palace, V.P. and Werner, J. (2006). Vitamin A and E in the maternal diet influence egg quality and early life stage development in fish: a review. Scientia Marina 70(2):41-57.

Petkam, R. and Moodie, G.E.E. (2001). Food particle size, feeding frequency, and the use of prepared food to culture larval walking catfish (*Clarias macrocephalus*). Aquaculture 194:349-362.

Phromkunthong, W. and Pipattanawattankul, A. (2005). Effect of *Spirulina* sp. on growth performance and antibody levels in hybrid catfish, *Clarias macrocephalus* × *Clarias gariepinus* (Burchell). Songklanakarin Journal Science Technology 27(1):115-132.

Promya, J. (2008) Assessment of Immunity Stimulating Capacity and Meat, Egg Qualities of Hybrid Tuptim Tilapia ND56 (*Oreochromis* sp.) Fed on Raw *Spirulina*. Ph.D. Thesis, Chiang Mai University.

Rym, B.D., G., Nejeh, T., Lamia, Y., Ali, C., Rafika, G., Khemissa, A., Jihene, O., Hela and Hatem, B.O. (2010). Modeling growth and photosynthetic response in *Arthrospira platensis* as function of light intensity and glucose concentration using factorial design. Journal Apply Phycology 22(6):745-752.

Santiago, C.B. and Gonzal, A.C. (1997). Growth and reproductive performance of the Asian catfish *Clarias macrocephalus* (Gunther) fed artificial diets. Journal Applied Ichthyology 13:37-40.

Shahidi, F., Metusalach and Brown, J.A. (1998). Carotenoid pigment in seafoods and aquaculture. Critical Reviews in Food Science 38(1):1-67.

Singh, S., B.N., Kate and Banerjee, U.C. (2005). Bioactive compounds from cyanobacteria and microalgae: an overview. Critical Rewiews in Biotechnology 25:73-95.

Sun, X., Chang, Y., Ye, Y., Ma, Z., Liang, Y., Li, T., Jiang, N., Xing, W. and Luo, L. (2012). The effect of dietary pigment on the coloration of Japanese ornamental carp (koi, *Cyprinus carpio* L.). Aquaculture 342-343:62-68.

Trabelsi, L., H.B., Ouada, H., Bacha and Ghoul, M. (2009). Combined effect of temperature and light intensity on growth and extracellular polymeric substance production by the cyanobacterium *Arthrospira platensis*. Journal of Applied Phycology 21:405-412.

Vonshak, A., S.M., Cheung and Chen, F. (2000). Mixotrophic growth modifies the response of *Spirulina* (*Arthrospira*) *platensis* (Cyanobacteria) cells to light. Journal of Phycology 36(4):675-679.

Welker, C., P.D., Negro and Sarti, M. (2001). Green algal carotenoids and yellow pigmentation of rainbow trout fish. Aquaculture International 9:87-93.

Zhang, X.-W., Y.-M., Zhang and Chen, F. (1999). Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of *Spirulina platensis*. Process Biochemistry 34:477-481.

(Received 29 July 2012; accepted 30 December 2012)