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Effect of Replacing Fishmeal with *Spirulina* on Growth, Carcass Composition and Pigment of the Mekong Giant Catfish

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Abstract: *Spirulina* has been commercially cultivated because of its high nutritional content. It can be considered a nutritional supplement for humans with various health benefits, and a feed supplement for animals having economic benefits. In aquaculture, *Spirulina* has been used to improve the immune system and increase the survival rate in juvenile commercial fish, as well as to increase their flesh pigment. From these applications, *Spirulina* could be used to replace high protein feed ingredient such as fishmeal and soybean meal to save the costs of feed. The Mekong Giant Catfish (Pla Buk), *Pangasianodon gigas* Chevey, is one of the largest species of freshwater catfish in the world. This fish is an endemic species in the Mekong River and its branches. At present, Thailand could culture this fish on a commercial scale. This study was conducted to investigate replacing fishmeal with *Spirulina*, at 0, 15, 30 and 100%, and to evaluate growth, feed utilization and pigment in flesh of fish. The results showed that the proximate composition of the various feeds (4 formulas) had similar nutritional value. Average daily gain, specific growth rate and feed conversion rate were not significantly different. However total biomass increase of fish fed with *Spirulina* 0% was significantly lower than fish fed with *Spirulina* 15, 30 and 100% ($p<0.05$). The standard length of the fish and weight in terms of flesh, gut and stomach were not significantly different. As well, flesh pigment and carotenoids were not significantly different.

Key word: Growth, mekong giant catfish, pigment, *Spirulina*

INTRODUCTION

Spirulina is a cyanobacterium that has been commercially cultivated for more than 10 years due to its high nutritional content; e.g. protein, amino acid, vitamin, minerals, essential fatty acid and b- carotene (Vonshak, 1997). *Spirulina* can be considered a nutritional supplement that has various health benefits for humans, and a feed supplement for animals having economic benefits. As an example it can be a suitable food supplement when fed to trout, sea bass, fancy carp, red tilapia, shrimp and mollusk. It has been found that the alga can be used as an alternative source of protein and can also be used to improve the color, flavor and quality of meat. Nowadays, *Spirulina* can be used to establish immune-potentiating functions in carp (Watanuki *et al*., 2006).

Mekong Giant Catfish, *Pangasianodon gigas* Chevey, is an important fish that inhabits the Mekong Basin. It has listed as being Critically Endangered in the 2005 IUCN red list of threatened species (IUCN, 2010). Recently, the artificial insemination and pond cultivation of the Mekong Giant Catfish has been successfully established. The Mekong Giant Catfish has been commercially bred, for which the raiser must consider many factors, such as requirements of investment and location because a vast and deep pond is needed, the high cost of feed, a shortage of the fry and the length of time needed for growth (around 5-6 years). There is a major group of giant catfish breeders within the country, as the market is only for domestic consumption (Manosroii *et al*., 2004).

To date, there is a limited amount of data that could be used to improve the growth of the Mekong Giant Catfish. There have been studies on the development of pelleted feed for fish that contains fine rice bran, broken-milled rice, fishmeal, dry algae, soybean meal, salt and multi-vitamins. However, there has been no clear data to indicate whether the effects of *Spirulina* additives for nutrient utilization can be beneficial for growth and whether there is an accumulation of caroteniods in flesh color and stomach content. As a result, the present study should provide information for the preparation of the pellet feed to maximize the productivity and growth of the

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Table 1: The ingredient-composition of each diet and total protein content on dry weight

Ingredients compostion of diets	T1 Protein (%)	T1 Weight (Kg)	T2 Protein (%)	T2 Weight(Kg)	T3 Protein (%)	T3 Weight(Kg)	T4 Protein (%)	T4 Weight (Kg)
Spirulina	-	-	0.8	1.5	1.5	3.0	14.0	28.0
Fish meal	16.5	29.0	15.4	27.0	14.8	26.0	-	-
Soybean meal	10.6	24.0	11.0	25.0	10.6	24.0	13.2	30.0
Rice bran	1.8	22.0	1.7	21.0	1.6	20.0	1.5	19.0
Broken-milled rice	1.5	25.0	1.5	25.5	1.6	27.0	1.4	23.0
Total protein	30	100	30	100	30	100	30	100

Mekong Giant Catfish. The objective of this research to evaluate the effect of feed containing various ratios of *Spirulina* on the growth of the Mekong Giant Catfish and to study the stomach content and carotenoid pigment in the flesh of the Mekong Giant Catfish that have been grown with feed containing various ratios of *Spirulina*.

MATERIALS AND METHODS

Experimental unit: Twelve ponds of 1x2x1 m (width x length x depth) was prepared at the Faculty of Fisheries Technology and Aquatic Resource, Maejo University.

Experimental animal: The fish were received from the Faculty of Fisheries Technology and Aquatic Resource, Maejo University. A total of 108 fish, the average weight of each fish before the experiment was 400±10 g and total length of 20±5 cm.

Experimental diets: The different feeding combinations (4 formulas of isoenergy diets, Table 1) were prepared as follows:

Diet 1: The combination of feed containing 100% fish meal (T1)
Diet 2: The combination of feed-stuff supplemented with 5% dried *Spirulina* powder (T2)
Diet 3: The combination of feed-stuff supplemented with 10% dried *Spirulina* powder (T3)
Diet 4: The combination of feed with 100% dried *Spirulina* powder (T4)

Preparation of experimental diet: Well mixed feeding materials were packed in plastic bags and kept in the refrigerator at -18ºC throughout the experiment. Micro-Kjeldahl methods were used to analyze protein content in feeds. (AOAC, 1995). Fish were fed two times each day at 3% of body weight per day (BW/day) and the feeding rates were adjusted fortnightly.

Experimental Place and time: This research was studied at the Faculty of Fisheries Technology and Aquatic Resource, Maejo University, Chiang Mai, Thailand for one year, 1 August 2008 - 30 July 2009.

Pigment analysis: 30 mL of 90% acetone was added to 0.02 g of flesh and it was then incubated for 30 min at room temperature. Then samples were centrifuged at 1,500 rpm for 5-10 min. After that 5 mL. of supernatant were added to the new tube and it was then distilled with 1 and 2 mL. of ethyl acetate to measure the absorbance at 472 nm (Foss *et al.*, 1984).

The comparison of pigment in flesh was done using the carotenoids colour fan.

Stomach content analyses: Stomach contents were sorted taxonomically and counted using a light microscope at 400X after sedimentation on slides. The volume of consumed items were determined by squashing the stomach contents on a slide and identification of algae, bacteria and fungi were determined through the use of identification books (Lin *et al.*, 2007).

Proximate composition: The fish carcasses was dried at 60ºC and blended, kept in desiccators jar. The analysis of the feed and the fish carcasses were done according to the following methods: protein was analyzed by micro-Kjeldahl Method; fatty acid was measured by dichloromethane extraction according to Soxlhet Method. The fiber was measured by fritted glass crucible method; ash was measured by burning the specimen in muffle furnace at 550ºC for 12 h and moisture content was measured according to the method of AOAC (1995) by oven heated treatment at 105ºC for 24 h.

Statistical analysis: The collected data were analyzed by Independent Samples t-test to statistically assess the treatment effects. t-tests was used for treatment comparisons at the statistically significant level of 0.05. All statistical analysis performed were done with SPSS version 11.5.

The water quality in experiment pond: The water quality in the experiment pond was monitored every month by recording the pH was measured by pH meter (Schott-Gerate CG 840), the conductivity level, dissolved oxygen using an oxygen meter (YSI Model 59). The nitrate-nitrogen and ammonia - nitrogen was analyzed using spectrophotometer (Hach DR/2000).

RESULTS AND DISCUSSION

The overall growth of the Mekong Giant Catfish: In this experiment, the feed consisted of isonitrogenous and

Table 2: Growth performance and feed utilizations of the Mekong Giant Catfish fed with the experimental diets

Growth	T1	T2	T3	T4
Total biomass increase /fish (g)	557.87±64.72^{b}	766.11±59.51^{a}	698.52±28.89^{a}	600±55.38^{a}
Average daily gain /day (g)	1.69±0.20	2.18±0.18	2.12±0.04	1.82±0.17
Specific growth rate (g)	0.20±0.01	0.21±0.01	0.21±0.01	0.21±0.01
Feed conversion rate	0.18 ±0.023	0.24±0.02	0.23±0.01	0.22±0.02

Values are mean + SEM. Values in the same row with different superscripts are significantly different ($p<0.05$)

Table 3: The weight and length of flesh, intestines and stomach and the standard length of the Mekong Giant Catfish

Treatment	T1	T2	T3	T4
Flesh weight (g)	373.33±21.80	410.00±26.454	26.67±17.63	340.00±50.33
Standard length (cm)	37.66±1.20	38.66±1.30	38.00±1.52	35.66±2.02
Intestine length (cm)	82.67±7.8	86.43±1.36	76.17±2.89	76.87±4.40
Intestine weight (g)	21.20±1.75	20.27±1.60	20.07±1.23	19.33±2.97
Stomach weight (g)	11.67±0.94	10.90±1.68	10.27±0.52	8.30±0.86

Values are mean + SEM. No significant difference was detected within each row ($p>0.05$)

Table 4: Proximate compositions (%) of flesh fed with the experimental diets (as dry weight basis)

Feeds	T1	T2	T3	T4
Protein (%)	18.81±0.33	20.71±1.02	20.82±0.20	21.54±0.72
Crude Fat (%)	12.15±0.59	12.03±0.20	10.85±0.59	12.05±0.38
Carbohydrate (%)	46.01±0.58	^{a}40.59±0.37^{b}	45.77±0.79^{a}	45.47±1.34^{a}
Fiber (%)	1.50±0.11^{b}	2.05±0.15^{a}	1.77±0.12^{ab}	1.78±0.01^{ab}
Ash (%)	10.32±0.18^{a}	11.69±0.22^{a}	11.41±0.71^{a}	8.42±0.15^{b}
Moisture (%)	11.21±0.24^{a}	12.93±0.39^{a}	9.38±0.13^{b}	10.74±0.98^{a}

Values are mean + SEM. Values in the same row with different superscripts are significantly different ($p<0.05$)

isocaloric feed in all treatments. These are shown in Table 1. In this study, the survival rate of the Mekong Giant Catfish was 100% on all diets. The growth rates of the Mekong Giant Catfish results are shown in Table 2. Total biomass increase of fish fed T1 was significantly lower than fish fed T2, T3 and T4 ($p<0.05$). The present research studied the effect of replacing 5, 10 and 100% of fishmeal by *Spirulina*. The highest weight yielded was found among the fish that were fed with the feed that contained *Spirulina* 5%, 766.11±59.51 g. Some studies have shown that feeding *Spirulina* to fish could improve their survival rate and growth rate (Belay *et al.*, 1996; Hayashi *et al.*, 1998). No significant differences were found in the average daily gain, specific growth rate and feed conversion rate ($p>0.05$). T2 produced a higher average daily gain and specific growth rate than fish fed with T1, T3 and T4. Previous research has shown that *Spirulina can be used* as a protein source in feeding two important fish in India, the Cata and the Rohu. *Spirulina* was mixed in the ratios of 25, 50, 75 and 100 %, respectively. It was found that the Rohu fish increased its growth, protein efficiency ratio, digestibility of dry matter, and both protein and lipid content in correlation with the amount of *Spirulina* consumed. They concluded that it was suitable to use *Spirulina* as a protein supplement source for both fish (Nandeesha *et al.,* 2001). These results showed that *Spirulina* could improve growth, reduction of mortality, overall elements of fish quality, firmness of flesh, brightness of skin color as well as improving the cost/performance ratio of the fish feed (Vonshak, 1997). The results from this experiment indicate that 5% dried *Spirulina* could be used to replace fishmeal and it yielded the highest weight and average daily gain/day.

The weight, length of flesh, intestines, stomaches and the standard length of the Mekong Giant Catfish: There were no significant differences in flesh-weight, weight and length of intestines, weight of the stomach and standard length of the Mekong Giant Catfish (Table 3). The flesh-weight was highest in T2. The intestine and stomach weights were highest in T1 and the standard and intestine lengths were highest in T2. These results show that the Mekong Giant Catfish fed with diet T2, which replaced 5% of fishmeal with *Spirulina*, could improve the optimal flesh-weight, as well as intestine and stomach weights.

Proximate compositions of the Mekong Giant Catfish flesh in this experiment are shown in Table 4. There were no significant differences in protein and crude fat in Mekong Giant Catfish flesh for all diets. The value of protein and crude fat ranged from 18.81-21.54 and 10.85-12.15%. The results show the percentage of protein in the flesh tended to increase with the increase of *Spirulina* in the T2-T4 diets, which were 20.71+1.02, 20.82+0.20 and 21.54+0.72%, respectively.

However, carbohydrate, fiber, ash and moisture did reveal significant differences. The carbohydrate content of flesh in the T2 group that were fed with 5% *Spirulina*, was the lowest value but the fiber, ash and moisture of flesh revealed the highest value. The results from this experiment show that the Mekong Giant Catfish which were fed with diet T2 gave a high level of protein with a low amount of carbohydrates in the flesh.

Table 5: The pigment in flesh and carotenoid content

Pigment	T1	T2	T3	T4
Carotenoid (mg /g fish)	1.01±0.58^{b}	0.89±0.56^{b}	0.51±0.25^{b}	1.52±0.73^{a}
Pigment in flesh (%)	9.66±0.88^{b}	10.00±0.57^{b}	10.33±0.33^{ab}	11.33±0.33^{a}

Values are mean + SEM. Values in the same row with different superscripts are significantly different (p<0.05)

Table 6: The alga found in the stomach of the Mekong Giant Catfish

Number (Cell/ml)	T1	T2	T3	T4
Chlorella sp.	4.00±0.58	4.33±0.33	4.67±0.88	3.33±0.33
Anabaena sp.	2.00±0.58	1.67±0.67	1.33±0.88	1.33±0.33
Scenedesmus sp.	1.00±0.48	0.33±0.13	0.67±0.37	1.67±0.58
Pediastrum sp.	0	0	0.33±0.03	1.00±0.06
Coelastrum Sp.	0.67±0.03	1.00±0.06	1.53±0.03	1.33±0.88

Values are mean + SEM. No significant difference was detected within each row (p>0.05)

The pigment in flesh and carotenoid content: The pigment in flesh and carotenoid content results are shown in Table 5. The highest levels of pigment in flesh and carotenoid content were found in T4. The results show that the Mekong Giant Catfish fed with *Spirulina* increasing diets gave an increase in pigment and carotenoid content. The present results support the previous studies, Lu *et al.* (2003), in which Tilapia were fed fresh *Spirulina*. The sensory evaluations of texture and color were better than fish fed with normal feed. The pigment compositions of *Spirulina* are phycocyanin, chlorophyll a and carotenoids. The carotenoid content is composed of xanthophyll (37%), b-carotene (28%) and zeaxanthin (17%) (Vonshak, 1997). *Spirulina* was used as feed to improve the pigmentation of Goldfish and Fancy Red Carp and to increase the Roche egg yolk color (Vonshak, 1997). This research was showed that, *Spirulina* could increase carotenoid content and pigment; this is supported with the study of Lu *et al.* (2003) in which Tilapia fed solely on raw *Spirulina, and* which gave high flesh quality suitable for sashimi. These results indicated that 100% w/w dried *Spirulina* powder increased both the pigment and carotenoid contents.

The stomach content in fish fed with experimental diets: The algae in the stomach of the Mekong Giant Catfish were found to include five species of alga (Table 6). There were no significant differences in the five species of alga. The 4 species of *Chlorophyta* were *Chlorella* sp., *Scenedesmus* sp., *Pediastrum* sp. and *Coelastrum* sp. One species of Cyanophyta was found to be *Anabaena* sp. These five species were found in ponds that are known to culture the Mekong Giant Catfish and proved to be the dominant species. The results of this experiment could confirm that Mekong Giant Catfish do eat the phytoplankton in ponds. The research on the stomach content was done in *Liza macrolepis* (Lin *et al.*, 2007). Stomach content in combination with stable isotopes was used to trace and compare the food source of large-scale mullet *Liza macrolepis* and other detritivorous fish species in sub-tropical mangrove creeks and a tropical lagoon in Taiwan. Volume of organic detritus always contributed > 50% of the stomach content of *L. macrolepis* in both habitats. Joint analyses of stomach content and stable isotopes indicated that benthic microalgae on sediment were the most important assimilated food in both seasons for the dominant detritivorous fish in mangroves, whereas a greater reliance on microalgae and macroalgae periphyton on oyster-culture pens was observed in the lagoon (Lin *et al.*, 2007). This data could be applied in our study of the biodiversity and habitat of the Mekong Giant Catfish in the Mekong River.

The water quality in the experiment ponds: The results of water quality in the experiment ponds found the pH to be between 6.75-7.05, the conductivity ranged from 96.40-100.52 µs/cm, dissolved oxygen was 7.80-8.42 mg/L, the nitrate-nitrogen was 1.53-2.25 mg/L, the ammonia - nitrogen ranged from 0.14-0.24 mg/L. Therefore, it was determined that water quality was suitable to culture Mekong Giant Catfish.

CONCLUSION

The results of the present study indicate that *Spirulina* could be incorporated in experimental feed for the Mekong Giant Catfish at up to 10% as a supplement to their feed. Not only did the total biomass, average daily gain and specific growth rate increase, but the flesh weights and standard length of the Mekong Giant Catfish were highest in T2 as well. The flesh weights were higher in T2 and the same as the standard length. It was shown that the Mekong Giant Catfish fed with the feed stuff supplemented with 5 and 10% *Spirulina* had increased both the production and growth performance (Nandeesha *et al.*, 1998, 2001).

However, the pigment in flesh and carotenoid content results were highest in T4.

In conclusion, *Spirulina* may be used as a supplement at 5 and 10% of fish meal in experimental feed for the Mekong Giant Catfish. These concentrations could improve the growth performance, specific growth rate and pigment of the Mekong Giant Catfish. Additionally, 100% of *Spirulina* could increase the highest carotenoid content and pigment in the flesh.

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