



**Full Length Article**

# The effects of *Spirulina platensis* and *Cladophora* Algae on the Growth Performance, Meat Quality and Immunity Stimulating Capacity of the African Sharptooth Catfish (*Clarias gariepinus*)

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## ABSTRACT

The effects of *Spirulina platensis* and *Cladophora* diets on growth performance, meat quality and immunity stimulating capacity in African Sharptooth Catfish (*Clarias gariepinus*) were studied. *S. platensis* and *Cladophora* sp. were used as supplementary feeds. Four experimental diets were set up including 0% algae + basal diets (Basal diets; BD), 3% *Spirulina* + basal diets (3% SD), 5% *Spirulina* + basal diets (5% SD) and 5% *Cladophora* + basal diets (5% CD) respectively. The initial size of the catfish was  $30.63 \pm 0.96$  -  $32.47 \pm 1.75$  g. They were stocked in  $30\text{ m}^2$  earthen ponds at a density of 30 fish/  $\text{m}^2$ . The African Sharptooth Catfish were raised for 60 days. The weight gain, specific growth rate and average daily growth of Catfish fed 5% CD, 5% SD and BD were significantly higher than those fed with 3% SD ( $p<0.05$ ). The carotenoid content in catfish fed 5% CD ( $2.37 \pm 0.15$   $\mu\text{g/g}$ ) were significantly higher than those fed with 5% SD, 3% SD and BD ( $p<0.05$ ) respectively. Fish fed with 5% SD had significantly ( $p<0.05$ ) higher immunity ( $1.70 \pm 0.10$  units/mL) than those fed with 3% SD, 5% CD and BD ( $p<0.05$ ), respectively. It can be concluded that the 5% CD feed could be used as catfish feed in order to enhance the carotenoid levels in the catfish, while the 5% SD feed is more suitable for increasing the immunity of the catfish. © 2011 Friends Science Publishers

**Key Words:** Catfish; *Spirulina platensis*; *Cladophora*; Immunity; Growth; Meat quality

## INTRODUCTION

The African Sharptooth Catfish (*Clarias gariepinus*) grow up to 1.17 m long and the adult fish weighs 10 - 25 kg. The male adult is slightly larger than the adult female (Napitapat, 2000). This catfish has yellow colored flesh and is a popular and delicious fish, especially in the Northern and Northeastern Regions of Thailand. Researchers have identified two types of algae as appropriate feed for the African Sharptooth Catfish: the *Spirulina platensis* and the *Cladophora*.

*Cladophora* is a member of the *Chlorophyta* group. It is a branching alga with attaching filaments that grow out of the back of the cell. Chloroplast is present in the *Cladophora* alga and pyrenoids are present throughout the plant's cells. Many nuclei are present in the cytoplasm and are surrounded by chloroplast (Wongrat, 2001). *Spirulina platensis* is a blue-green spiral coiled alga, with the spiral varying among different species (Promya *et al.*, 2008). The optimum N: P ratio of kitchen wastewater (Kw) and oil-extracted fermented soybean water (Sw) for cultivation of *S. platensis* was evaluated. The highest levels of biomass production of *S. platensis* were achieved using 5%Sw (0.90 g/L) and Zm (0.80 g L<sup>-1</sup>) with an N:P ratio of 6:1 and 6.1:1,

respectively. The highest levels of  $\beta$ -carotene and C-phycocyanin in *S. platensis*, were achieved in cultures with 10%Sw (0.37 mg/g and 21.27 mg g<sup>-1</sup>, respectively (Promya *et al.*, 2008).

The *Spirulina* alga is rich in protein and vitamins, and can be used to improve the immunity capacity of the animals which consume it. Consumption of *Spirulina* alga also increases the ability to absorb nutrients. When *Spirulina* alga is used as feed for young prawns and fingerlings, the fish exhibit good coloring, as well as maintain a low death rate and a high growth rate (Sermwattanakul & Bamrungham, 2000). Previous studies using the raw *Spirulina* alga as the primary feed given to the Nile tilapia feed have found an increase in reproductive, birth and survival rates, compared with conventional fish feed. These studies have also found an increase in the amounts of linoleic acid, gamma-linolenic acid (GLA), protein and an improved color in the meat of the fish compared with fish fed on standard instant feeds (Lu & Takeuchi, 2003). The 3% *Spirulina* supplemented in pellet feed and hormone application improved growth and maturation performance of brood stock *Pangasius* catfish (Meng-Umphan, 2009). Previous studies demonstrated their economic benefits when *S. platensis* and *Cladophora* algae

were fed to the African Sharptooth catfish for a period of 2 months. The effects of this diet on the growth performance, meat quality and immunity stimulating capacity of fish were observed.

This experiment aimed to determine, which feed mixture would yield the optimal results, as well as to accumulate a set of base information, which could be used for further studies. The objectives of this study to determine the effects of using different amounts of *Spirulina* and *Cladophora* algae mixed with standard feed on flesh color, carotenoid levels, growth characteristics, immunity stimulating capacity in the African Sharptooth Catfish and its effect on water quality.

## MATERIALS AND METHODS

**Preparation of materials:** This included construction of 12 fish ponds (dimensions  $5 \times 6 \times 1$  m), which were firstly dug and cleaned. Lime ( $\text{CaCO}_3$ ) was then used to line the empty ponds (50-100 kg/rai) and left for 7 days. After that water from a well was used to fill the ponds to a depth of 0.8 m and fertilizer was added (250 kg/rai) in order to stimulate plankton growth.

**Preparation of experimental fish:** A total of 10,800 African Sharptooth Catfish were procured from the Faculty of Fisheries Technology and Aquatic Resources, Maejo University. The average weight of these fish was 12 - 15 g/fish. They were raised in a  $5 \times 6 \times 1$  m basket-like pond and fed with the control diet (BD) for 2 weeks, receiving the feed twice a day at 8:30 a.m. and 4:30 p.m. Observations were made about the fish's appetites and the health of the fingerlings was checked. There were no diseases found. After this, 900 fish of similar size were selected and placed in a  $5 \times 6 \times 1$  m pond (fish density = 30 fish/ $\text{m}^2$ ).

**Spirulina and Cladophora algae preparation:** *Spirulina* samples were obtained from the Faculty of Fisheries Technology and Aquatic Resources, Maejo University and propagated in a  $2.5 \times 15 \times 0.20$  m raceway pond using 20% efficient microbes (EM) animal manure liquid culture mixed with some chemicals (Promya & Saetun, 2005). The cell density of the experimental algae started at the optical density (OD) 0.30 using a DR 2000 Spectrophotometer with light wavelength of 560 nanometers. After 10 ten days growth, the OD was measured as 0.8-1. At this point, the alga was harvested, dried, and ground into a powder to be used in the experiment. *Cladophora* algae were grown at the Faculty of Fisheries Technology and Aquatic Resources, Maejo University in a  $2.5 \times 15 \times 0.15$  m earthen pond. The earthen pond was filled with tap water mixed with some chemicals. The algae starter samples covered about 20% of the surface area of the earthen pond. After a period of 10-15 days, the algae had covered the entire surface area. It was then harvested, dried and ground into a powder then mixed with standard fish feed in varying amounts as listed in Table I. The samples were baked (dried in oven) at a temperature of 60°C for 12-15 h until they were completely dry before

putting in bags and stored at room temperature (Promya & Saetun, 2005).

**Experiment plan:** A Completely Randomized Design (CRD) was implemented using four treatments with three replicates, while standard instant fish feed was procured at an animal feed supply stores and then was mixed with the *Spirulina* and *Cladophora* algae as follows:

Treatment 1 (BD): instant standard feed mixed with 0% algae (cost = 26.06 Baht/kg).

Treatment 2 (3% SD): instant standard feed mixed with 3% *Spirulina* algae (cost = 32.06 Baht/kg), Treatment 3 (5% SD): instant standard feed mixed with 5% *Spirulina* algae (cost = 36.06 Baht/kg), Treatment 4 (5% CD): instant standard feed mixed with 5% *Cladophora* algae (cost=27.32 Baht/kg).

The four diets from the above treatment were analyzed at the Faculty of Fisheries Technology and Aquatic Resources, Maejo University for protein content, fat content, moisture content, ash content, fiber content, carbohydrate content, nitrogen free extract (NFE) content, energy content and carotenoid contents according to AOAC (1990). The results are shown in Table I.

**Data collection:** Before putting the 900 experimental catfish in the fish ponds, baseline data was measured and collected for the following categories: Carotenoid levels in the catfish flesh, growth, red blood cell count, white blood cell count and immunity stimulating capacity. All experimental catfish were then placed in each of the 12 fish ponds ( $5 \times 6 \times 1$  m). The initial average weights of the catfish specimen were  $30.63 \pm 0.96$  -  $32.47 \pm 1.75$  g/fish. The catfish were fed twice a day at 8:30 a.m. and 4:30 p.m. 3-5% of the fish were tested for weight each day and the amount of feed was recorded each week throughout the 2 months of the experiment period. The water quality was tested every 15 days, in order to maintain appropriate water quality levels. Catfish growth rates were measured using the methods described in Boyd and Tucker (1992). Water temperature, pH level, total alkalinity levels, dissolved oxygen (DO) levels, ammonia levels and orthophosphate-phosphorus levels were measured and adjusted throughout the experiment.

**Growth analysis:** Behavioral and physical characteristics observations were made by the assigned staff throughout the experimental period. Observations were made about general health signs and eating habits and manner, as well as the cleanliness of the water. Each time the fish pond was cleaned, 20% of the pond's volume was drained and then replaced with new water. Fish weight gain data was collected by sampling 10% of the total fish in each pond and calculating the average weight every 2 weeks. After that, calculations were made to determine values for weight gain, specific growth rate, average daily growth, feed conversion rate, protein efficiency ratio, survival rate and total investment according to the following formulas:

$$\text{A. (Weight gain; %)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

B. (Specific growth rate; % /day) is equal to:  $SGR = \frac{(In \text{ Fish weight at end of experiment} - In \text{ Fish weight at the beginning of experiment})}{\text{Number of days during experiment}} \times 100$

C. Average daily growth; g/fish/day is equal to:

$$\frac{(\text{Fish weight at end of experiment} - \text{Fish weight at the beginning of experiment})}{\text{Number of days during experiment}}$$

D. Feed Conversion Rate (unit) is equal to:  $FCR = \frac{\text{Weight of feed}}{\text{Increase of fish weight}}$

E. Protein efficiency ratio (unit) is equal to:  $PER = \frac{\text{Increase in fish weight}}{\text{Amount of protein consumed}}$

F. Survival rate (%) is equal to:  $\frac{\text{Number of surviving fish} \times 100}{\text{Number of initial fish}}$

G. Total investment needed to achieve production (variable) = Feed cost + Fingerlings costs + Labor costs.

**Chemical analysis:** Analyses of specified feed ingredients presented in the fish meat were conducted as pre-and post-experiment analyses. This process consisted of collecting 10% of the experimental catfish from each pond and drying them in an oven for 48 h at 60°C. The fish meat was then examined for moisture content, protein content, fat content, fiber content, ash content, carbohydrate content and the nitrogen free extract (NFE) levels according to the AOAC (1990) method.

Measurements of carotenoid levels in feed and fish meat were taken using a 20 g. feed sample and 10-15% of the total fish from each fish pond. The fish meat was processed into fine pieces and then dried in an oven for 12 h at 60°C. This meat was pounded into a fine powder and divided into 20 g samples. Both the feed and fish meat samples were then examined for carotenoid levels using the method according to Sommer *et al.* (1992).

**Red and white blood cell counts and immunity stimulating capacity:** To study the ability to stimulate the immune capacity, a lysozyme activity assay was conducted according to the methods described by Sardar *et al.* (2001). Briefly, the dry *Micrococcus* sample (0.2 mg mL<sup>-1</sup>) in a 0.04 M phosphate buffer saline solution (pH 5.75) was used as the substrate where 40 µL of serum from the fish sample was added to 3 mL of the bacteria. The opacity levels at 540 nm decreased after placing the sample at room temperature for 0.5 and 5 min were measured. A unit of lysozyme activity is referred to as the opacity level has decreased 0.001 min<sup>-1</sup>. To measure the red and white blood cell counts, a blood sample was taken from the catfish after 60 days of rearing and a slide was prepared from this sample. This sample was put in a fixative reagent to stabilize the blood cells. At this point, it is necessary to quickly close the sample to avoid contamination. The red blood cells will then change their shape and the sample can produce a Wright-Giemsa stain. This will last for one minute and then needs to be added to a Phosphate buffer test tube for one minute and then washed with distilled or tap water before being dried. The sample can then be examined under a microscope to count the number of blood cells.

**Statistical analysis:** The effects of using the *Spirulina* and *Cladophora* algae feed were measured through weight gain, specific growth rate, average daily growth, protein efficiency ratio, survival rate, investment/output ratio, carotenoid levels, fish meat quality, red and white blood cell counts, immunity stimulating capacity, water quality and chemical levels in water in the experimental ponds. The data from the measurements were analyzed by variance analysis (ANOVA) in order to determine the effects and different of treatment using Duncan test at  $p \leq 0.05$ . The SPSS program was used.

## RESULTS

**Growth parameters:** At the start of the experiment (0 Days), the average body weight of the catfish was  $30.63 \pm 0.96$ - $32.47 \pm 1.75$  g/fish. The catfish gained weight after the start of the experiment and differences between the four treatments appeared by Day 15. At this point, catfish being fed with 5% SD weighed more than those being fed with BD, 3% SD and 5% CD. At Day 30 and Day 45, the catfish being fed with 5% CD weighed more than the catfish receiving the other types of feed. At the end of the experiment on Day 60, the fish being fed with BD, 5% SD and 5% CD weighed significantly ( $p < 0.05$ ) more than the fish receiving 3% SD (Fig. 1).

The weight gain, specific growth rate and average daily growth rate were higher for the catfish fed with BD, 5% SD and 5% CD than for the catfish being fed 3% SD ( $p < 0.05$ ) (Table II). There was no difference among the four treatments for the survival rate and feed conversion rate. The survival rate for all four treatments of feed was  $76.22 \pm 12.68$ - $78.78 \pm 8.86\%$  and the feed conversion rate was  $(1.07 \pm 0.21$ - $1.32 \pm 0.43)$  (Table II).

**Chemical and dietary attributes:** The protein efficiency ratio was higher ( $p < 0.05$ ) for the catfish receiving BD, 5% SD and 5% CD than for the catfish being fed 3% SD (Table III). The investment output ratio was lower for the catfish being fed with BD and 5% CD than for the catfish being fed with 3% SD and 5% SD ( $p < 0.05$ ) (Table III). The carotenoid levels in the catfish prior to the experiment showed no statistical difference among the four feed-treatments and ranged between  $0.62 \pm 0.05$ - $0.65 \pm 0.04$  µg/g. For the post experiment measurements, the carotenoid levels were statistically significantly higher ( $p < 0.05$ ) for the catfish being fed with 5% CD than for the groups being fed with BD, 3% SD and 5% SD (Table III; Fig. 2).

The protein levels in the fish receiving BD, 5% SD and 5% CD were significantly ( $p < 0.05$ ) higher than the protein levels in the fish being fed with 3% SD (Table IV). The carbohydrate contents and ash contents were not significantly different among the four feed treatments. The fat content in the Catfish receiving 3% SD and 5% SD was significantly higher than those being fed with BD and 5% CD ( $p < 0.05$ ). The moisture content in the Catfish receiving 3% SD was significantly ( $p < 0.05$ ) higher than those being

**Table I: Proximate compositions (% dry weights) of experimental diets**

Diet Type	Protein (%)	Carbohydrate (%)	Fat (%)	Moisture (%)	Ash (%)	Fiber (%)	Energy kcal/kg	Carotenoid in flesh (µg/g)
0% algae	29.70	38.05	8.97	9.45	8.95	4.88	2778.00	3.25
3% <i>Spirulina</i>	30.30	40.43	7.23	10.34	7.96	3.74	2716.50	18.58
5% <i>Spirulina</i>	30.90	41.52	8.42	9.84	6.63	2.69	2675.50	32.60
5% <i>Cladophora</i>	29.80	36.06	9.06	9.86	9.41	5.81	2660.30	48.50

**Table II: Average growth rates, survival rates, and feed conversion rates of African Sharptooth Catfish for each feed type during the 60 day experimental period**

Diet type	Weight gain (%)	Specific Growth Rate (%/day)	Average Daily Growth (g/fish/day)	Survival Rate (%)	Feed Conversion Rate (Units)
0% algae (BD)	422.59±43.10 <sup>a</sup>	2.39±0.16 <sup>b</sup>	2.16±0.29 <sup>a</sup>	76.22±12.68 <sup>ns</sup>	1.07±0.21 <sup>ns</sup>
3% <i>Spirulina</i> (3% SD)	359.11±11.20 <sup>b</sup>	2.14±0.03 <sup>b</sup>	1.81±0.09 <sup>b</sup>	78.00±3.85 <sup>ns</sup>	1.32±0.43 <sup>ns</sup>
5% <i>Spirulina</i> (5% SD)	459.44±21.02 <sup>a</sup>	2.53±0.08 <sup>a</sup>	2.47±0.03 <sup>a</sup>	76.44±5.09 <sup>ns</sup>	1.24±0.21 <sup>ns</sup>
5% <i>Cladophora</i> (5% CD)	452.47±8.74 <sup>a</sup>	2.50±0.03 <sup>a</sup>	2.45±0.12 <sup>a</sup>	78.78±8.86 <sup>ns</sup>	1.09±0.08 <sup>ns</sup>

**Table III: Protein efficiency ratio, investment and pre-and post-carotenoids levels per feed type**

Diet Type	Protein Efficiency Ratio (Units)	Investment Output Ratio (variable) (kg/Baht)	Carotenoids in meat Pre-experiment (µg/g)	Carotenoids in meat Post-experiment (µg/g)
0% algae (BD)	4.32±0.57 <sup>a</sup>	20.63±3.54 <sup>c</sup>	0.64±0.04 <sup>ns</sup>	5.10±0.10 <sup>d</sup>
3% <i>Spirulina</i> (3% SD)	3.62±0.18 <sup>b</sup>	37.85±11.52 <sup>b</sup>	0.65±0.04 <sup>ns</sup>	10.70±0.10 <sup>c</sup>
5% <i>Spirulina</i> (5% SD)	4.95±0.06 <sup>a</sup>	57.33±9.14 <sup>a</sup>	0.65±0.04 <sup>ns</sup>	12.03±0.15 <sup>b</sup>
5% <i>Cladophora</i> (5% CD)	4.89±0.25 <sup>a</sup>	20.23±1.33 <sup>c</sup>	0.62±0.05 <sup>ns</sup>	15.37±0.15 <sup>a</sup>

**Table IV: Average levels of nutrients in catfish flesh after 60 days**

Diet Type	Protein (%)	Carbohydrate (%)	Fat (%)	Moisture (%)	Ash (%)	Fiber (%)
0% algae (BD)	35.93±0.90 <sup>a</sup>	4.07±0.22 <sup>ns</sup>	31.44±2.30 <sup>ab</sup>	7.28±0.32 <sup>c</sup>	18.81±0.54 <sup>ns</sup>	2.47±0.57 <sup>a</sup>
3% <i>Spirulina</i> (3% SD)	31.00±1.0 <sup>c</sup>	4.69±0.45 <sup>ns</sup>	34.88±1.59 <sup>a</sup>	9.23±0.49 <sup>a</sup>	18.60±0.53 <sup>ns</sup>	1.60±0.50 <sup>ab</sup>
5% <i>Spirulina</i> (5% SD)	33.00±0.03 <sup>a</sup>	5.27±1.42 <sup>ns</sup>	34.09±1.82 <sup>a</sup>	8.27±0.39 <sup>b</sup>	18.50±1.32 <sup>ns</sup>	0.87±0.31 <sup>b</sup>
5% <i>Cladophora</i> (5% CD)	37.18±1.28 <sup>a</sup>	4.57±0.41 <sup>ns</sup>	27.99±4.23 <sup>b</sup>	8.15±0.45 <sup>b</sup>	20.33±1.52 <sup>ns</sup>	1.77±0.57 <sup>ab</sup>

**Table V: Red and White Blood cell and lysozyme activity assay counts for each feed test case during the 60 day experimental period**

Measurement	BD	3% SD	5% SD	5% CD
Number of red blood cells (60 days) (x 10 <sup>6</sup> cell/ uL)	2.65±0.03 <sup>c</sup>	2.68±0.04 <sup>b</sup>	2.87±0.06 <sup>a</sup>	2.68±0.04 <sup>c</sup>
Number of red blood cells (60 days) (x 10 <sup>3</sup> cell/ uL)	76.10±2.00 <sup>c</sup>	86.33±0.58 <sup>b</sup>	95.33±3.06 <sup>a</sup>	78.33±8.14 <sup>c</sup>
Lysozyme activity assay (Units/mL) (60 days)	0.90±0.10 <sup>c</sup>	1.30±0.10 <sup>b</sup>	1.70±0.10 <sup>a</sup>	1.17±0.06 <sup>b</sup>

**Table VI: Fish pond water quality and chemistry**

Feed Type	Temperature (°C)	pH	Alkalinity (mg L <sup>-1</sup> )	DO (mg L <sup>-1</sup> )	NH <sub>3</sub> -N (mg L <sup>-1</sup> )	PO <sub>4</sub> -P (mg L <sup>-1</sup> )
BD	28.17 ± 0.15 ns	7.10 ± 0.20 ns	85.67±1.53 <sup>ns</sup>	3.97 ± 0.21 ns	0.09±0.001 <sup>ns</sup>	0.003±0.006 <sup>ns</sup>
3% SD	28.23 ± 0.06 ns	7.13 ± 0.42 ns	87.00±1.00 <sup>ns</sup>	4.10 ± 0.36 ns	0.08±0.002 <sup>ns</sup>	0.003±0.001 <sup>ns</sup>
5% SD	28.17 ± 0.15 ns	6.80 ± 0.30 ns	85.00±1.53 <sup>ns</sup>	4.50 ± 0.30 ns	0.07±0.001 <sup>ns</sup>	0.004±0.001 <sup>ns</sup>
5% CD	28.13 ± 0.15 ns	6.90 ± 0.10 ns	84.33±3.06 <sup>ns</sup>	4.33 ± 0.21 ns	0.07±0.002 <sup>ns</sup>	0.004±0.001 <sup>ns</sup>

Note: Different letters (a,b,c,d) show significant statistical differences (p<0.05), ns=no significant difference

fed with BD, 5% SD and 5% CD. The fiber content in the Catfish receiving BD was significantly (p<0.05) higher than those being fed with 3% SD, 5% SD and 5% CD (Table IV).

**Red and white blood cell counts and immunity stimulating capacity:** Fingerlings, which received feed 5% SD had higher values for red and white blood cell counts and the lysozyme activity assay after 60 day experiment period than those that received feeds BD, 3% SD and 5% CD (Table V).

**Fish pond water quality and chemistry:** There was no significant difference among the four treatments for water temperature (ranging 28.13 ± 0.15 – 28.23 ± 0.06°C) or in the water pH levels (ranging 6.80 ± 0.30 – 7.13 ± 0.42). There was no significant difference between the four feed treatments in the alkalinity level (ranging 84.33 ± 3.06 – 87.00 ± 1.00 mg L<sup>-1</sup>) or in the dissolved oxygen (DO) level (ranging 3.97 ± 0.21 – 4.50 ± 0.30 mg L<sup>-1</sup>). There was no significant difference between the four feed treatments in the ammonia (NH<sub>3</sub>-N) level (ranging 0.07 ± 0.001 – 0.09 ±

0.001 mg L<sup>-1</sup>) or in the orthophosphate-phosphorus (PO<sub>4</sub>-P) level (ranging 0.003 ± 0.006 - 0.004 ± 0.001 mg L<sup>-1</sup>) (Table VI).

## DISCUSSION

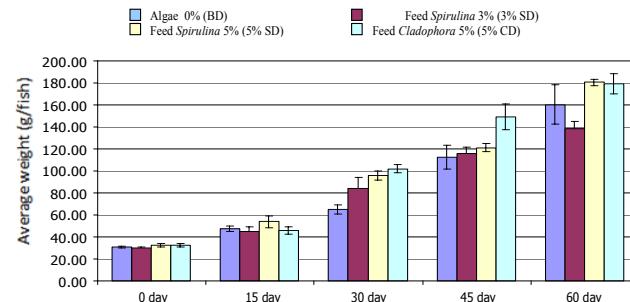
This research concluded that catfish fingerlings fed with BD, 5% SD and 5% CD diets showed favorable growth (weight gain, specific growth rate, average daily growth, protein efficiency ratio & protein levels in fish meat) when compared with the fingerlings received the 3% SD feed. The survival rates did not vary significantly among the four feed types, but the investment output ratio and feed conversion rates were favorably higher for BD and 5% CD than for 3% SD and 5% SD feed types. Red tilapia fish that were fed with a diet of standard feed mixed with 10% *Spirulina* alga had a higher growth rate and weight gain than the control group (Lu & Hiroo, 2004). The results of this research are similar to those found by Promkunthong and Pipattanawattankhul (2005) in which catfish fed with a diet of standard feed mixed with 10% *Spirulina* alga had the highest protein efficiency ration and the most carotenoids in their meat.

Another study was conducted in which 10-15% *Spirulina* alga replaced crushed fish in the feed given to Red Tilapia fish in order to bring the protein level up to 30% and this yielded the highest average daily growth rate and specific growth rate, as well as an increase in the carotenoid levels in the Tilapia meat (Promya *et al.*, 2003). Similar research was done by Promya and Hongwittayakorn (2003) in which *Spirulina* and *Cladophora* algae were fed to Fancy Carp fingerlings. This study found that the fish received the *Spirulina* powder demonstrated a higher average daily growth rate, weight gain rate, survival rate, and post experimental carotenoid level in meat than the Fancy Carp that were fed with standard feed and feed mixed with *Cladophora*.

Research conducted by Duncan and Klesius (1996) found that *Spirulina* alga was a good source of protein for animal feed, as well as containing high amounts of vitamins and minerals. Besides this, the cellular structure of *Spirulina* alga is easily digestible and does not contain cellulose. Different levels of *Spirulina* alga can be mixed with feeds according to the eating behaviors of the fish and differing abilities to digest the protein from plant sources.

The present study found that fish fed with 5% SD exhibited higher red and white blood cell counts and a higher immunity stimulating capacity (measure by a lysozyme activity assay). The red blood cell counts were especially significantly higher for the fish receiving 5% SD, which was similar to the findings of Terry *et al.* (2000) that Striped Tilapia fed with alga had a red blood cell count of 1.91-2.83 X 10<sup>6</sup> cell/µL. The increase in red and white blood cells and immunity stimulating capacity could be due to the presence of C-phycocyanin in the *Spirulina* alga, which can help build the immunity capacity (Vonshak,

**Fig. 2: Carotenoids in the fish meat after experiment completion. It was found that the flesh color of the catfish receiving feed 5% CD was more yellow than the catfish receiving the other types of feed**



**Fig. 1: Average weight (g/fish) of African Sharptooth Catfish during 60 day experimental period**



1997). Both *Spirulina* and *Cladophora* algae contain carotenoids, which affect the health of fish, specifically improving health and increasing the ability to fight off infections through the reduction of stress levels (Nakono *et al.*, 2003).

The water parameters in experimental ponds with fish receiving algae in the feed had higher values of pH, alkalinity and nutrients (especially NH<sub>3</sub>-N) than the ones in the ponds with fish not receiving any algae supplements. The higher level of nutrients in ponds with fish receiving algae could be the result of absorption of materials in the pond water. When the algae fall into the ponds, they changes the water quality. However, the water in all experimental ponds were cleaned by partially draining and replacing with new water regularly in order to maintain the water quality with the standard range necessary for raising fish, including pH level between 6.5 - 9, DO value not lower than 5 mg L<sup>-1</sup>, alkalinity level between 100 - 120 mg L<sup>-1</sup> as CaCO<sub>3</sub>, PO<sub>4</sub>-P value between 0.1 - 0.5 mg L<sup>-1</sup> and NH<sub>3</sub>-N level not higher than 0.05 mg L<sup>-1</sup> (Boyd & Tucker, 1992).

In conclusion, it is possible to use *Cladophora* and *Spirulina* algae as fish meal replacement or protein supplements in feeding of catfish. When adding an amount

of algae equal to 5% of the dry feed weight, it will increase growth rate, protein conversion rate and survival rates. *Cladophora* alga contains carotenoids, which increase the yellow color in the catfish flesh, and *Spirulina* alga will increase the red and white blood cells counts and improve the immunity capacity of the catfish.

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