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Communication

Production of Generation-2 Mekong giant catfish (*Pangasinodon gigas*) cultured with *Spirulina sp.*

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Abstract: The purpose of this study is to evaluate the treatment of *Spirulina*-supplemented pellet feed to 5-year-old F1 groups of Mekong giant catfish (*Pangasinodon gigas*) from the brood stock and intended for use as breeders. The effects on their growth and maturation when cultured in an earthen pond were observed. Results revealed that, compared to control, there was more gain in weight while the feed conversion ratio was lower. The number of red blood cells was also higher while that of white blood cells was lower, compared to control. Out of 18 treated fish (9 males and 9 females), 6 males and 2 females gave sperms and eggs while none from control group did. It was concluded that *Spirulina* supplemented in pellet feed can improve growth and maturation performance to the brood stock of Mekong giant catfish.

Keywords: *Pangasinodon gigas,* brood stock, breeding, *Spirulina*

Introduction

The annual catch of the Mekong giant catfish (*Pangasinodon gigas* or Pla Buek in Thai) in Mekong River at Chiang Kong district in Chiang Rai province of Thailand registered, during 1986-2007, a maximum of 65 fish in 1990. However, in later years (2000 and 2003-2006) this number declined to 1-2, and zero (in 2001, 2002 and 2007) (Figure 1) [1]. This giant catfish in the Mekong River may thus become extinct in the near future. The Mekong giant catfish plays an important role as a valuable fish for the people along the Mekong River. This fish bred in captivity in several rivers, lakes and ponds need at least 10-15 years to reach maturation [1].

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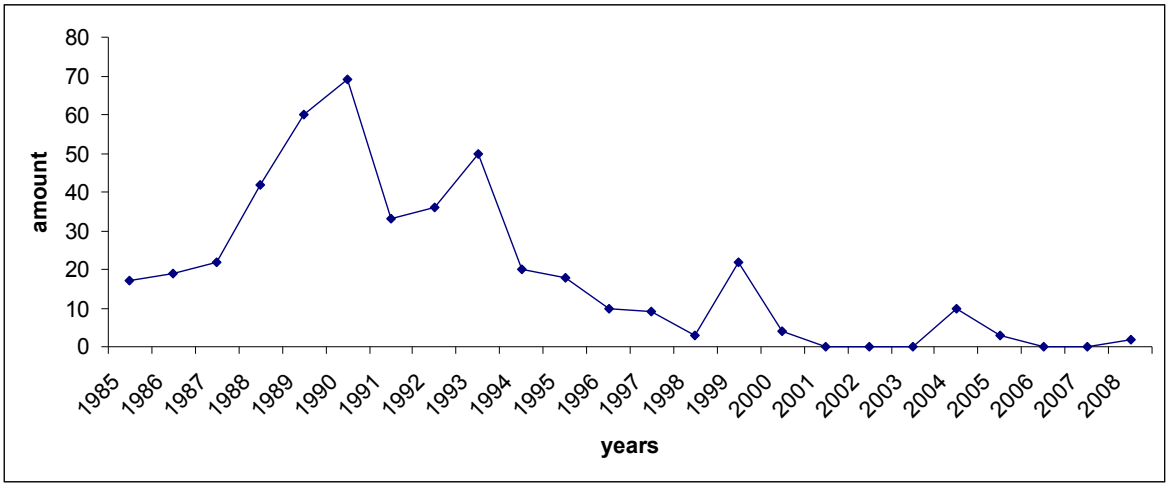

Figure 1. Annual catch of Mekong giant catfish from the Mekong River

Production of quality fry entails the production of good breeders. Since one of the constraints in raising this species is production cost, the feed aspect should be taken into consideration to optimist growth and reproduction with the least possible cost. Of the microalgae and cyanobacteria that have been used as foodstuffs, food supplements and animal feed in many parts of the world, the *Spirulina sp*. (SP) is the most popular [2]. Among its beneficial properties are its antioxidant [3] and immunostimulant [4] activities and a high protein content. It has been found that raw SP when fed to tilapia produces good flesh quality and gives slightly better sensory evaluations for colour, texture and fattiness [5].

Results of studies utilising SP as sole feed or additive in pellet diet to assess the reproductive performance of different animal species have been variable. The present study is made in order to evaluate the growth performance, blood levels (red and white blood cells), and the number of brood stock of *P. gigas* prior to breeding. To our knowledge, this study has not been conducted before and its outcome should be beneficial for commercial and conservation purpose.

Materials and Methods

Brood stock and feed preparation

A total of thirty-six 5-year-old *P. gigas* (Figure 2) with an average weight of 6 kg were randomly treated by one of two treatments with three replications (six fish per replicate) for 8 months (January-August, 2007) in a 55-m^2 pen in an earthen pond. In treatment 1 (T1) the fish were fed with ordinary formulated pellets (control), while in treatment 2 (T2) they were fed with pellets which had been incorporated with fresh SP at an amount of 9%. The composition of each formulation of the pellets (Table 1, Figure 3) was analysed by an approximate method.

Data collection

The gain in weight (GW), feed conversion ratio (FCR) and biomass were obtained monthly. Samples of blood were obtained in March for read blood cell (RBC) and white blood cell (WBC) count

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using a hemocytometer (Figure 4). In August, the fish brood stocks were checked for sperms and eggs by stripping at the abdomen. The number of mature brood stock was counted in each treatment.

Figure 2. *Pangasinodon gigas*, 5 years old

Table 1. Components of feed for each treament

Ingredient (% crude protein)	Treatment 1 (control)			Treatment 2 (SP incorporated)		
	Raw material (Kg.)	Protein (%)	Energy (KJ/g)	Raw material (Kg)	Protein (%)	Energy (KJ/g)
Fishmeal (61%)	16	9.7	274.42	7	4.27	120.06
Spirurina (58%)	0	0	0	9	5.22	169.62
Soybean (44%)	40	17.6	604.54	40	17.6	604.54
Rice brand (8%)	21	1.68	322.25	21	1.68	322.25
Broken rice (6%)	21	1.26	271.53	21	1.26	271.53
Oil (0%)	2	0	16.94	2	0	16.94
Total	100	30.3	1,489.68	100	30.03	1,504.94

SP 0% SP 9%

Figure 3. Fish pellets with and without 9% SP

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Figure 4. Blood sampling

Statistical analysis

The GW, FCR, RBC and WBC with standard errors were presented in tables and graphs. The variation of treatment was analysed by analysis of variance (ANOVA, $P<0.05$). Significant differences between treatments were analysed using Duncan's Multiple Range Test (DMRT) by SPSS software.

Results and Discussion

GW and FCR

As shown in Table 2 and Figures 5-6, T2 (with 9% SP) gave GW of 872±34 grams/fish whereas the valud for T1 (control) was 711±107 grams/fish. Thus, T2 attained about 20% increase in GW as compared with the control. However, statistical analysis revealed no significant difference. In Figure 5, the FCR was 1.64±0.23 for T1 and 1.33±0.07 for T2, which was lower than that for T1, but statistically no significant difference was observed. In a study by Promkhun-thong and Unchalee [6], they observed an increase in weight, specific growth rate, FCR and antibody level in *Clarias sp.* supplemented with 10% SP in the feed. Kithja [7] also observed an increasing tendency in weight, specific growth rate and FCR when *Pangasius bocourti* was supplemented with SP, although no significant differences were observed. Pariwannta [8] reported a FCR level of 2.27 to 2.59 for *P. bocourti* fed with SP and stocked at different densities.

Table 2. Statistics of the average weight gain, feed conversion ratio, and red and white blood cells from each treatment

Item	Treatment 1 (no SP)	Treatment 2 (9% SP)
Gain in weight (gram)	711 ± 107^a	872 ± 34^a
Feed conversion ratio	1.65 ± 0.13^a	$1.34 \pm 0.0\ 4^a$
Red blood cells (cells/mm^3)	$920{,}833 \pm 518{,}461^a$	$995{,}833 \pm 115{,}470^a$
White blood cells (cells/mm^3)	$2{,}167 \pm 381^a$	$1{,}583 \pm 803^a$

Note: Figures that have the same superscript letter are not statistically different ($P>0.05$.) among treatment means.

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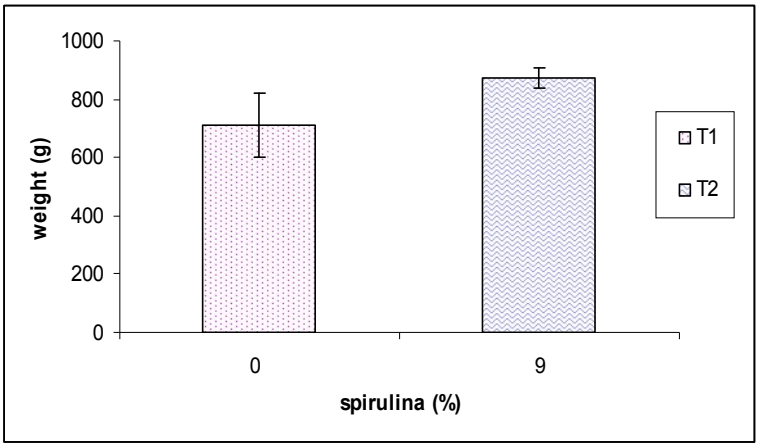

Figure 5. Weight gain/fish from each treatment

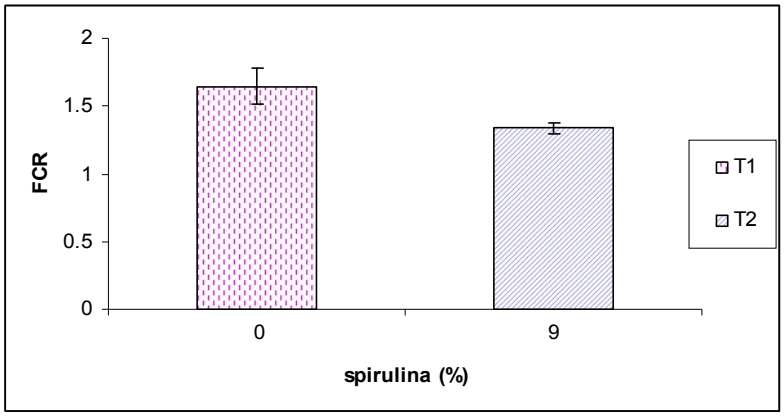

Figure 6. Feed conversion ratio from each treatment

RBC and WBC

As shown in Table 2 and Figures 7-9, although no significant differences were observed between the 2 treatments in terms of RBC and WBC, the higher RBC count was found in T2 (995,833 ±115,470 cell/mm^3) while T1 (control) registered a lower count (920,833 ±518,461 cell/mm^3). The WBC, on the other hand, was higher in T1 (2,166 ±381 cells/mm^3) than T2 (1,583 ±803 cells/ mm^3). Kithja [7] observed that the supplementation of SP in the feed for *P. bocourti* resulted in an increase of red and white blood cell count. *Spirulina* has a dark blue-green colour because it is rich in a brilliant blue polypeptide called phycocyanin. Studies have shown that phycocyanin affects the stem cells found in the bone marrow. Stem cells are the "grandmother" of both the white blood cells that make up the cellular immune system and the red blood cells that oxygenate the body [7].

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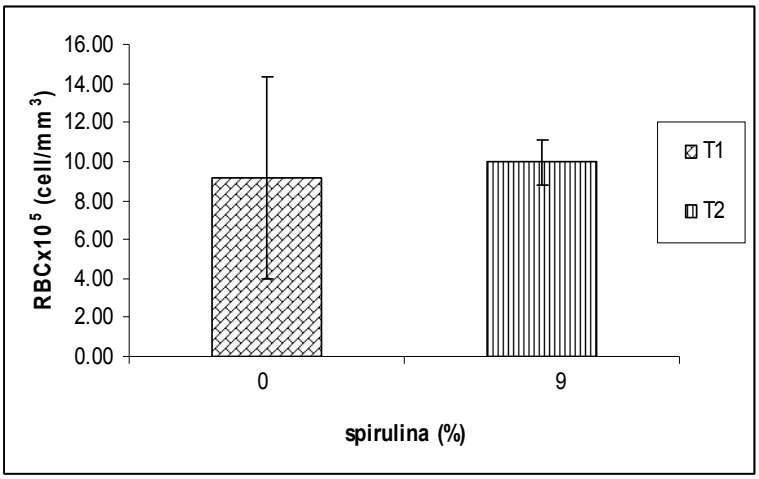

Figure 7. RBC count from each treatment

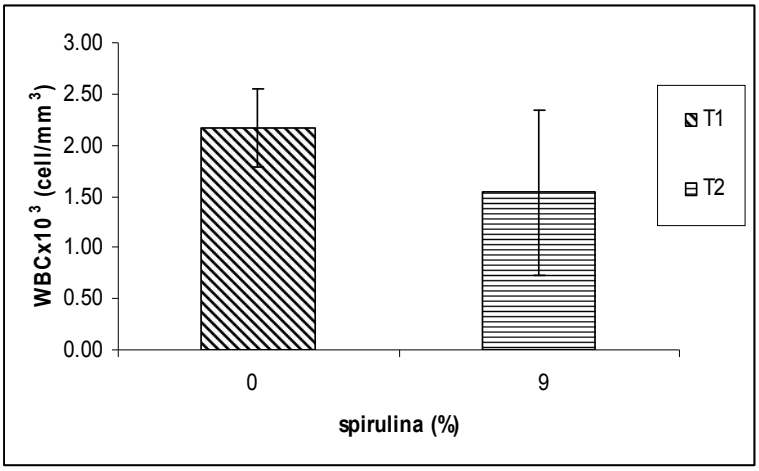

Figure 8. WBC count from each treatment

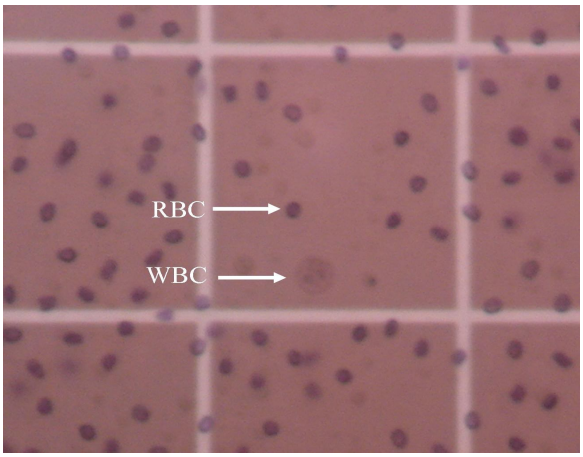

Figure 9. RBC and WBC

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Maturation (collection of sperms and eggs)

As shown in Table 3 and Figures10-12, 2 females with eggs and 6 males with sperms were obtained from treatment 2, while none was obtained in treatment 1. The 300 fingerlings (F2) from treatment 2 were also obtained by artificial fertilisation. Pariwannta [8] also observed an increase in the sex hormone level (estradiol) when the feed for *P. bocourti* contained 3% SP. Fecundity in gilthead sea bream (*Sparus aurata*) was found to significantly increase with an increase in dietary level of n-3 HUFA (polyunsaturated fatty acids with 20 or more carbon atoms) up to 1.6% [9]. Other nutrients which have been shown to affect fecundity include vitamin E and ascorbic acid [10].

Table 3. Number of male and female brood stock with sperms and eggs from each treatment (recorded in August 2007)

Treatment	Total number	Number of males	Number of females	Number with sperms	Number with eggs
T1 (control)	18	7	11	-	-
T2 (9% SP)	18	9	9	6	2

Figure 10. Collection of sperms

Figure 11. Collection of eggs

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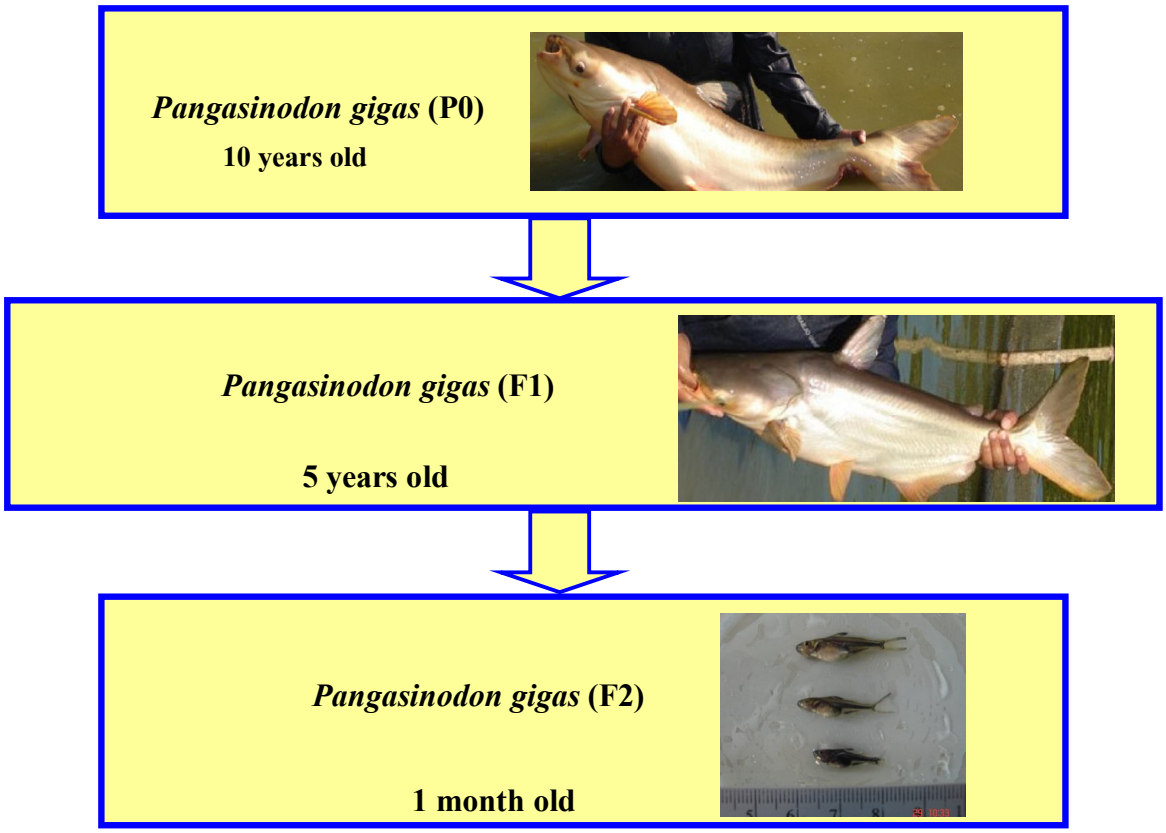

Figure 12. The brood stock of Makong giant catfish from parent (P0) to generation 2 (F2) in earthen pond

Conclusions

Feeding *P. gigas* resulted in a higher GW, RBC and number of mature brood stock. This further suggests that supplementing SP in the feed for this species of fish can have a significant benefit in their reproduction. Our further study will focuss in quantifying both the estradiol and testosterone levels in separate males and females. The quantity and quality of the milt and oocytes will also be taken into consideration.

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